From DEPT. MICROBIOLOGY, TUMOR AND CELL BIOLOGY
Karolinska Institutet, Stockholm, Sweden

PRO-SENESCENCE THERAPY BY TARGETING MYC AND ITS NETWORK AS A STRATEGY TO COMBAT CANCER

Fan Zhang

Karolinska Institutet

Stockholm 2019

Published by Karolinska Institutet.
Printed by Eprint AB 2019

ISBN 978-91-7831-397-6

Pro-senescence therapy by targeting MYC and its network as a strategy to combat cancer

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Fan Zhang

Principal Supervisor:
Professor Lars-Gunnar Larsson
Karolinska Institutet
Department of Microbiology, Tumor and cell biology

Co-supervisor(s):
Assistant professor Alina Castell
Karolinska institutet
Department of Microbiology, Tumor and cell biology

Opponent:
Professor Jonas Nilsson
University of Gothenburg
Department of Sahlgrenska Cancer Center

Examination Board:
Professor Staffan Strömblad
Karolinska institutet
Department of Biosciences and Nutrition

Professor Marene Landström
Umeå University
Department of Wallenberg Centre for Molecular Medicine

Associate Professor Andreas Lundqvist
Karolinska institutet
Department of Department of Oncology-Pathology

The public defense of this thesis will take place in Atrium, Nobels väg 12 A, Karolinska institutet, Solna

Tuesday, 16th April, 2018 at 9.30

To My Family

ABSTRACT

Cancer is defined as abnormal cell proliferation with the potential of metastasis and is an increasing threat to global health as the population becomes older. Cancer is triggered by gain-of-function mutations in oncogenes and loss of function mutations in tumor suppressor genes. This leads to the breakdown of two important barriers against tumorigenesis-cellular senescence, which is permanent cell cycle arrest, and apoptosis, which is a programmed cell death. *MYC* is one of the classical oncogenes and its deregulation is observed in various types of cancer where it plays an essential role in tumor development. It has been more than three decades since MYC was discovered; however, the precise role of MYC in normal cells and in different types of cancer is still under intense research and debate. For example, it has been shown that MYC can overcome senescence induced by another oncogene, RAS, and RAS can override apoptosis triggered by MYC, leading to oncogenic transformation of primary rodent cells. However, there is no evidence indicating to which extent, this cooperation between MYC and RAS occurs also in human primary cells, since transformation of human cells requires several steps in addition to overcoming senescence and apoptosis.

Therefore, in Paper I, to investigate cooperation between MYC and RAS in human primary cells, we used human BJ fibroblast cells where we established a double inducible RAS and MYC system (BJ-RAS-MYC). In this system RAS expression is induced by doxycycline while MYC is expressed as a MycER fusion protein controlled by 4-hydroxytamoxifen (OHT) treatment. As expected, RAS induction triggered cellular senescence, as evidenced by increased senescence-associated β-galactosidase (SA-β-GAL) activity, enlarged cell size, arrested cell proliferation, increased expression of histone H3 lysine 9 trimethylation (H3K9me3), 21 and p16, and decreased phosphorylation of pRB. MYC induction preferentially triggered apoptosis, measured as increased cell death and cleaved PARP. Dual induction of RAS and MYC decreased H3K9me3 and SA-β-GAL but enhanced p16 level compared to RAS induction alone, suggesting MYC failed to completely overcome RAS-induced senescence. On the other hand, MYC-trigged cell death was not rescued by RAS induction, even when MYC activity was tuned down or MYC activation was scheduled at different times after RAS induction. Thus, MYC and RAS failed to cooperate to overcome each other's fail-safe mechanisms, in which the main obstacle seemed to be MYC-induced cell death. Given the importance of p53 in regulation of apoptosis and that p53 was triggered upon MYC induction in our system, we knocked down p53 to try to rescue cells from cell death. Unexpectedly, p53 depletion (BJ-RAS-MYC-shp53) failed to overcome MYC-induced cell death although it rescued RAS-induced senescence. In conclusion, MYC and RAS did not cooperate to abrogate apoptosis and senescence, respectively, in human primary cells, even after p53 depletion, suggesting that additional oncogenic events are required to overcome senescence and apoptosis in this system.

In has been reported that cancer treatments such as chemotherapy and radiation can trigger senescence in tumor cells. Hence, the concept of pro-senescence therapy has emerged as an alternative of anti-cancer therapy, especially for tumors resistant to apoptosis-inducing therapies or where conventional therapies cause severe side-effects. In Paper II, we explored the potential of pro-senescence therapy in melanoma treatment. First we investigated the efficacy of eleven clinical and preclinical drugs as monotherapy in a senescence screen, using a panel of melanoma cell lines with different driver or mutations, such as $BRAF^{V600E}$, $NRAS^{Q61R}$, PTEN, PI3K, p53 and CDK4. Following treatment, cell number, nuclear/cell size, EdU incorporation, intensity of H3K9me3, p53 as well as HLA class I staining was measured using an Olympus scanR high-content imager system. We found that vemurafenib and trametinib induced senescence in some but not all BRAF-mutated cell lines, while palbociclib, crizotinib and BKM120 induced senescence most of cell lines irrespective of BRAF/NRAS mutation status. These results were confirmed using a additional

senescence markers, such as analysis of SA-β-GAL activity, cell cycle distribution and phosphorylation of pRb. Moreover, palbociclib and BKM120 also increased expression of several SASP factors and palbociclib and crizotinib enhanced expression of HLA class I, implying the potential of increased immunogenicity.

We next addressed the question whether combination treatment could synergize with vemurafenib to overcome intrinsic and acquired vemurafenib resistance. Therefore, we performed a combination treatment senescence screen, combining vemurafenib and trametinib with the other drugs. The results showed that palbociclib, crizotinib and BKM120 synergize with vemurafenib to trigger senescence in both vemurafenib-sensitive and –resistant cells. In addition, we found that the combination of palbociclib with crizotinib further enhanced senescence including SASP factors of relevance for immune responses, again independent of BRAF/NRAS mutation status. We also looked at expression of molecules associated with recognition by T and NK cells. Palbociclib increased the expression of HLA class I and HLA class II, but also of PD-L1 and the NKG2D ligands ULBP 2/5/6 in several melanoma cell lines, and the expression of these molecules were in some cases further enhanced by combining palbociclib with crizotinib. This indicates that palbociclib induced both positive and negative immune markers for $CD8^+$ T cell and NK cell recognition in melanoma cells, implying possible benefit of using anti-PD-1 combination treatment to eliminate such cancer cells.

Although it is clear that MYC is one of the most important players in tumorigenesis, there are, unfortunately, no selective MYC inhibitors available in clinic today. The main reason for this is that transcription factors like MYC are difficult to target due to their lack enzymatic activity and intrinsically disordered features. Since MYC function is dependent on interaction with its partner MAX, this motivates us to try to identify and characterize MYC inhibitors directly targeting the MYC:MAX interaction. Thus, in Paper III and Paper IV, we used a cell-based bimolecular fluorescence complementation (BiFC) screen, where MYC and MAX were fused with two parts of yellow fluorescent protein (YFP), only the close proximity of MYC and MAX can restore the fluorescent signal. We found six compounds (MYCMI-2, MYCMI-6, MYCMI-7, MYCMI-9, MYCMI-11 and MYCMI-14) potentially targeting the MYC-MAX interaction. This was validated in cells using the following assays: 1) split Gaussia luciferase (GLuc), where MYC and MAX are fused with two complementary fragments of GLuc; 2) in situ proximity ligation assay (isPLA), in which after primary antibodies binding, secondary antibodies conjugated with oligo were bound to the primary antibodies, and the close proximity assisted oligos ligation and followed by PCR, fluorescent probe to PCR products can be visualized; 3) U2OS-MycER system showed us that these compounds repressed MYC target genes. Among the six compounds, MYCMI-6 and MYCMI-7 exerted the highest efficacies. We next investigated whether these molecules directly target MYC:MAX interaction *in vitro* using recombinant proteins in microscale thermophoresis (MST) assay, which is based on changes in the mobility of a fluorescent molecule in a temperature gradient upon binding of ligands/inhibitors). We also used surface plasmon resonance (SPR), which is a highly sensitive assay to determine the affinity between protein and ligand and to measure the kinetics of interactions. With both these assays, MYCMI-6 and MYCMI-7 were confirmed to inhibit MYC:MAX interaction via directly binding to MYC bHLHZip domain in the low micromolar range, which is more potent than previously reported MYC:MAX inhibitors. A difference between MYCMI-6 and MYCMI-7 was that the former did not affect MYC expression, while MYCMI-7 decreased MYC protein, but not mRNA, level. The mechanism behind this is not clear. Furthermore, both MYCMI-6 and MYCMI-7 inhibited growth and induced apoptosis in a number of MYC-dependent tumor cell lines, such as *MYCN*-amplified neuroblastoma and MYC-driven Burkitt's lymphoma. MYCMI-6 reduced tumor cell proliferation in a mouse xenograft model of MYCN-

amplified neuroblastoma. In addition, MYCMI-7 reduced tumor burden in mouse models of MYC-driven acute leukemia, triple negative breast cancer and MYCN-amplified neuroblastoma, and prolonged survival in the latter two models.

It has been reported previously that MYC depletion by shRNA induced cellular senescence in melanoma with BRAF or NRAS mutation, suggesting that MYC inhibition could be used in pro-senescence therapy. In Paper V, we evaluated the senescence-inducing potential of the MYC inhibitors MYCMI-6 and MYCMI-7, reported in Paper III and Paper IV, as well as the previously reported MYC:MAX inhibitor 10058-F4 (F4) and one BET inhibitor JQ1, which represses MYC transcription, in a panel of melanoma cell lines. In a senescence screen performed as in Paper II, MYCMI-7 triggered senescence in the majority of cell lines, and was more potent than F4. This was further validated in selected melanoma cells with $BRAF^{V600E}$ mutation, and the effects of F4, MYCMI-7 and JQ1 was compared. JQ1 mainly induced senescence; MYCMI-7 a mix of apoptosis and senescence and F4 preferentially triggered apoptosis. Among the three MYC inhibitors, only MYCMI-7 exerted a persistent proliferation inhibitory effect after drug removal. We also performed a senescence screen for the combination of the MYC inhibitors and the $BRAF^{V600E}$ inhibitor vemurafenib, MEK inhibitors (trametinib/selumetinib) and the CDK4/6 inhibitor palbociclib. The combination treatment of MYC inhibitors with vemurafenib/trametinib/selumetinib synergistically induced senescence and overcame vemurafenib/trametinib/selumetinib resistance. In addition, MYC inhibitors synergized with palbociclib to induce senescence in $BRAF^{V600E}$- mutated cells. Thus, combining MYC inhibitors with BRAF, MEK and CDK4/6 inhibitors synergistically induced malignant melanoma senescence.

The data presented in this thesis has shed further light on the function of MYC in normal and tumor cells, but also opening up the possibility of targeting MYC in pro-senescence therapy.

LIST OF SCIENTIFIC PAPERS

I. **Fan Zhang**, Siti Mariam Zakaria, Vedrana Högqvist Tabor, Madhurendra Singh, Susanna Tronnersjö, Jacob Goodwin,Galina Selivanova, Jiri Bartek, Alina Castell & Lars-Gunnar Larsson
MYC and RAS are unable to cooperate in overcoming cellular senescence and apoptosis in normal human fibroblasts
Cell cycle, 2018, volume 17, page 2679-2715.

II. **Fan Zhang**, Ishani Das, Sofi Eriksson, Jeroen Melief, Madhurendra Singh, Marina Stantic, Alireza Azimi, Michelle Da Silva Liberio, Jacob Goodwin, Rainer Tuominen, Fredrik Jerhammar, Suzanne Egyhazi Brage, Johan Hansson, Rolf Kiessling, Galina Selivanova, Margareta Wilhelm, Klas Wiman, and Lars-Gunnar Larsson
Pro-senescence therapy - a new strategy to overcome drug resistance and enhance immunorecognition of malignant melanoma cells
Manuscript, 2019.

III. Alina Castell, Qinzi Yan, Karin Fawkner, Per Hydbring, **Fan Zhang**, Vasiliki Verschut, Marcela Franco, Siti Mariam Zakaria, Wesam Bazzar, Jacob Goodwin, Giovanna Zinzalla & Lars-Gunnar Larsson
A selective high affinity MYC-binding compound inhibits MYC:MAX interaction and MYC-dependent tumor cell proliferation
Scientific reports, 2018.

IV. Alina Castell, Qinzi Yan, Karin Fawkner, **Fan Zhang**, Malin Wickström, Wesam Bazzar, Marcela Franco, Cecilia Dyberg, Per Hydbring, Vasiliki Verschut, Linnéa Schmidt, Suman Vodnala, Mårten Fryknäs, Lars Johansson, Sören Lehmann, Cecilia Krona, Sven Nelander, John Inge Johnsen and Lars-Gunnar Larsson
MYCMI-7 - a small MYC-binding compound that inhibits MYC:MAX interaction and tumor cell growth in culture and *in vivo* in a MYC-dependent manner.
Manuscript, 2019.

V. **Fan Zhang**, Michelle Da Silva Liberio, Jacob Goodwin, Rolf Kiessling, Galina Selivanova, Alina Castell and Lars-Gunnar Larsson
MYC inhibitors work synergistically with BRAF, MAPK and CDK inhibitors to block malignant melanoma cell growth by induction of cellular senescence
Manuscript, 2019.

CONTENTS

LIST OF ABBREVIATIONS

4EBP1	eIF4E binding protein 1
AML	Acute myeloid leukemia
AMPK	AMP-activated protein kinase
APCs	Antigen-presenting cells
ASF1a	Antisilencing function 1a
ATM	Ataxia-telangiectasia-mutated
AURKA	Aurora kinase A
bHLHZip	Basic helix-loop-helix/leucine zipper
BiFC	Bimolecular fluorescence complementation
C/EBPβ	CCAAT/enhancer binding protein-β
CAFs	Cancer-associated fibroblasts
CAK	CDK-activating kinase
CCFs	Cytoplasmic chromatin fragments
CCL2	C-C motif chemokine ligand 2
CD47	Cluster of differentiation 47
CDKs	Cyclin-dependent kinases
ChIP	Chromatin immunoprecipitation
CHK2	Checkpoint kinase 2
CML	Chronic myeloid leukemia
CTD	C-terminal domain
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
CXCL8	Chemokine (C-X-C) motif ligand
CXCR2	CXC chemokine receptor 2
DCs	Dendritic cells
DDR	DNA damage response
DISC	Death-inducing signaling complex
DNMT3a	DNA methyl transferase 3a
dNTP	Deoxyribonucleoside triphosphate
EdU	5-ethynyl-2’-deoxyuridine
EGF	Epidermal growth factor

ER	Estrogen receptor
ERK	Extracellular signal-regulated mitogen-activated protein kinase
ETC	Electron transport chain
EZH2	Enhancer of zeste 2
FADD	FAS-associated protein with death domain
FGF	Fibroblast growth factor
GAP	GTPase activating proteins
GPCR	G-protein coupled receptors
GSK3β	Glycogen synthase kinase 3 beta
H3K9me3	Histone H3 lysine 9 trimethylation
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HDR	Homology directed repair
HIRA	Histone repressor A
HKMT	Histone lysine methyltransferase
HLA	Human leukocyte antigens
ICAM-1	Intercellular adhesion molecules-1
IFN-γ	Interferon-γ
IL-12	Interleukin 12
IL-1RA	IL-1a receptor antagonist
IMPDH2	Inosine monophosphate dehydrogenase 2
isPLA	in situ proximity ligation assay
JAK/STAT	Janus kinase/signal transducers and activators of transcription
LAG-3	Lymphocyte activation gene-3
MAGE	Melanoma antigen-encoding
MAPK	Mitogen activated protein kinase
MCM	Minichromosome maintenance
MDM2	Mouse double minute 2 homolog
MDSCs	Myeloid-derived suppressor cells
MEFs	Mouse embryonic fibroblasts
MEK	Mitogen-activated protein kinase kinase

MHC	Major histocompatibility complex
MIZ-1	MYC-interacting Zn finger protein-1
MNT	Max's next tango
MST	Microscale thermophoresis
mTOR	Mammalian target of rapamycin
NF1	Neurofibromin 1
NF-κB	Nuclear factor-κB
NHEJ	Non-homologous end joining
NK	Natural killer
NLS	Nuclear localization signal
NuRD	Nucleosome remodeling deacetylase
OHT	4-hydroxytamoxifen
OIS	Oncogene-induced senescence
PAK	p21-activated protein kinase
PCA	Protein-fragment complementation assay
PDAC	Pancreatic ductal adenocarcinoma
PDGFR	Platelet-derived growth factor receptor
PDK1	Pyruvate dehydrogenase kinase 1
PD-L1	Programmed death-ligand 1
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol-4, 5-bisphosphate
PIP3	Phosphatidylinositol-3, 4, 5-triphosphate
PKM2	Pyruvate kinase M2
PML	Promyelocytic leukemia
pRB	Retinoblastoma protein
pre-RC	Prereplicative complex
PRPS2	Phosphoribosyl pyrophosphate synthetase 2
PTEN	Phosphatase and tensin homologue
REFs	Rat embryonic fibroblasts
RNAP II	RNA Polymerase II
ROS	Reactive oxygen species

RPS14	Ribosomal protein S14
RTK	Tyrosine kinase receptors
rtTA	Reverse tetracycline-controlled transactivator
SADS	Senescence-associated distension of satellites
SAHF	Senescence-associated heterochromatin foci
SASP	Senescence-associated secretory phenotype
SA-β-GAL	Senescence-associated β- galactosidase
SCLC	Small cell lung carcinomas
SHH	Sonic hedgehog
SIRTs	Sirtuins
SKP2	S-phase kinase associated protein 2
SOS	Son of sevenless
SPR	Surface plasmon resonance
TFIIH	Transcription factor IIH
TGF-β	Transforming growth factor beta
TILs	Tumor infiltrating lymphocytes
TIS	Therapy-induced senescence
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRE	Tet response element
T_{reg} cells	Regulatory T cells
tRNA	Transfer RNA
TS	Thymidylate synthase
TSS	Transcription start site
TSG	Tumor suppressor gene
VEGF	Vascular endothelial growth factor
YFP	Yellow fluorescent protein

1 INTRODUCTION

1.1 CANCER

Cancer is defined as abnormal cell proliferation with the potential to invade into other parts of the body and has a major impact on society of the whole world. According to NIH statistics, in 2018, 1735 350 new cases of cancer are estimated to be diagnosed in the Unites States and 609 640 patients will die from cancer (Siegel et al., 2018). Cancer is a genetic disease caused by mutations although epigenetic changes also play a role. Some types of cancer are hereditary due to germline mutations, and these often have an early onset. However, most types of cancer are due to somatic mutations and affect adults as a result of aging, stochastic events as well as environmental and other factors. From a molecular point of view, a normal cell is maintained in a homeostatic state due to several well-tuned self-control systems, involving a number of cell signaling pathways, sophisticated regulation of the cell cycle, different fail-safe mechanisms responding to different types of stress and a preset limited lifespan. The human body is built up by numerous different cell types that need to communicate with each other, and cell of the immune system adds an extra protective role to the whole organism. However, due to genetic and epigenetic aberrations, this state of equilibrium can become destabilized and the protection systems can be compromised, eventually leading to cancer development. Thus, in the first part of introduction, I will briefly present the concept of oncogenes and tumor suppressor genes, relevant cell signaling pathways, cell cycle regulation, cellular fail-safe mechanisms – apoptosis and senescence, tumor immunology, some tumor types of relevance for this thesis and current cancer therapies. As the second part, I will focus on senescence – one of the topics of this thesis – in relation to its biology and importance to cancer therapy. The last part will be dedicated to one of the best-known oncogenes, MYC - the other main topic of the thesis- in terms of its structure, regulation, biological functions and targeting strategies.

1.1.1 Oncogenes and tumor suppressor genes

The famous discovery by Peyton Rous validated the possibility of a sarcoma in the domestic fowl transmitted to other fowl, which was the first discovery that tumor can be triggered by certain tumor-inducing agent after isolation and transduction, later termed as oncogenes (Rous, 1910, 1911). Oncogenes were first identified in avian and murine retroviruses, e.g. SRC, ERBB2, RAS and MYC. Later, researchers found cellular homologs of these oncogenes, which were named “proto-oncogenes”. Proto-oncogenes normally promote cell growth and division under a tight control, however, after mutation, they become oncogenes with the potential to cause tumors. Besides the previously mentioned receptor/non-receptor tyrosine kinases, small GTPases and transcription factors, there are also other types of genes promoting tumor initiation and progression, including programmed cell death regulators such as BCL-2 and mouse double minute 2 homolog (MDM2). Their oncogenic functions can be triggered by genetic alterations affecting either their protein level or structures or both. For example, it has identified ERBB2 oncogenic function via its amplification or kinase domain

mutation, leading to it ligand-independent constitutive activation (Moasser, 2007). Therefore, oncogenes promote tumor progression with a gain of function.

On the other hand, there are also another important group of genes suppressing tumor development, therefore named tumor suppressor genes (TSG). Tumor suppressor genes can function in different ways. They can work as transcription factors, such as p53 and retinoblastoma protein (pRB), and repress proliferation-related genes thereby arresting the cell cycle. Another type of TSGs are involved in activation of the DNA damage response (DDR) and DNA repair, for example BRCA1 and BRCA2. Moreover, they can trigger programmed cell death or dampen survival signals, such as FAS and phosphatase and tensin homologue (PTEN). In cancer, mutations in tumor suppressor genes cause loss of function.

1.1.2 Cell signaling

Cells respond to external triggers mainly through cell-surface receptors, which can transmit external signals into the cell interior via activation of different signaling pathways. There are different groups of cell-surface receptors, such as tyrosine kinase receptors (RTK), G-protein coupled receptors (GPCR), NOTCH receptors and etc. RTKs, as an example, are mobile laterally in the plane of the plasma membrane as monomers. Extracellular mitogens (epidermal growth factor (EGF), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), etc) can bind to corresponding RTKs (EGF receptor, FGF receptor and VEGF receptor, etc) and trigger their dimerization, leading to trans-phosphorylation of the intracellular domains of the receptors at tyrosine residues. Phospho-tyrosine can act as a binding site for intracellular proteins containing SH2 domains, leading to activation of downstream effectors, by which signals will be amplified and trigger different responses, such as cell proliferation, survival and migration. For example, in the presence of the growth factor EGF, the RTK EGFR is activated leading to recruitment of the adaptor protein GRB2 and the guanine nucleotide exchange factor “son of sevenless” (SOS), which activates downstream RAS-signaling. With the assistance of SOS, GDP bound to RAS is released and replaced by GTP, leading to the activation of RAS GTPase activity. RAS in turn activates the downstream mitogen activated protein kinase (MAPK) pathway by disrupting RAF’s internal autoinhibitory mechanism by altering its conformation, leading to RAF phosphorylation by membrane-associated kinases, such as p21-activated protein kinase (PAK) and SRC, followed by activation of RAF serine/threonine-specific protein kinase function. This leads to the initiation of protein kinase cascade, including activation of mitogen-activated protein kinase kinase (MEK) and extracellular signal-regulated mitogen-activated protein kinase (ERK). Eventually ERK phosphorylates and activates many transcription factors, such as MYC and c-FOS, leading to transcription of proliferation-related genes and promotion cell proliferation (Adams et al., 2000; Leone et al., 1997; Sears et al., 1999; Sears et al., 1997; Shaulian and Karin, 2001). Therefore, MAPK pathway plays an essential role in cell proliferation. Moreover, GTPase activating proteins (GAPs), such as neurofibromin 1 (NF1), can replace GTP to GDP bound to RAS, causing the inactivation of RAS. Thus, NF1 is

considered as a tumor suppressor and over 1400 NF1 loss-of-function mutations are identified in cancer without (Ratner and Miller, 2015).

In addition to the MAPK pathway, RAS also activates phosphoinositide 3-kinase (PI3K) pathway. PI3K are heterodimeric molecules located in the plasma membrane. PI3K phosphorylates its lipid substrate phosphatidylinositol-4, 5-bisphosphate (PIP2) to phosphatidylinositol-3, 4, 5-triphosphate (PIP3). The latter can attract and activate the serine/threonine kinase AKT. Upon its activation, AKT phosphorylates downstream effectors involved in many biological processes. For example, glycogen synthase kinase 3β (GSK3β) is phosphorylated and inactivated by AKT. GSK3β is involved in many processes, such as promoting degradation of the WNT pathway downstream effector β-catenin and of MYC, as well as negatively regulating eukaryotic initiation factor eIF2B. Therefore, inactivation of GSK3β leads to derepression of the above-mentioned factors and subsequently promotion of cell proliferation. AKT, on the other hand, phosphorylates and inactivates pro-apoptotic protein BAD, leading to inhibition of apoptosis. Moreover, AKT can also influence protein synthesis through the activation of the mammalian target of rapamycin (mTOR) kinase, which accelerates protein translation. Therefore, the PI3K/AKT pathway favors cell survival, enhances cell proliferation, reduces apoptosis and increases cell growth. PTEN, which is a phosphatase preferentially dephosphorylating phosphoinositide substrates, e.g. PIP3, is a negative regulator of the PI3K/AKT pathway. This explains why genetic inactivation of PTEN commonly occurs in different types of cancer (Milella et al., 2015).

1.1.3 Cell cycle

Cell proliferation is under a tight control by the cell cycle, during which a series of biochemical events are taken place. The cell cycle is divided into four distinct phases: the G1 phase, during which cells increase in size and prepare for DNA synthesis; S phase, during which DNA replication occurs; G2 phase, a preparation phase for cell division; M phase, mitosis, during which chromosomes condense and segregate followed by cytokinesis when two daughter cells are formed. After cell division, cells can either reenter the cell cycle or exit the cell cycle into the G0 phase, where cells are quiescent until they are activated by another wave of growth factors.

There are a variety of regulatory and operative proteins to assist this process. Firstly, the cyclin/cyclin-dependent kinases (CDKs) complexes, which are the ‘engines’ driving the cell cycle. The cyclins - a family of proteins that act as positive regulatory subunits controlling the CDKs - work in an autonomous, oscillating manner, varying in level throughout the cell cycle. Their partner proteins, the CDKs, is a group of protein kinases that modify substrates involved in cell cycle, are relatively constant in level, but require cyclins to bind and activate their catalytic functions. Since cyclins’ synthesis and breakdown define the precise timing of a specific stage, different cyclin-CDKs complexes are present and function differently in the cell cycle. During a typical cell cycle, cyclin D-CDK4/6 initiates the progression through the G1 to the S phase, and cyclin D breaks down in the beginning of S phase. Meanwhile, cyclin

E is synthesized at late G1 phase, binds to and activates CDK2 to function during late G1 and early S phase. Then cyclin A-CDK2 is formed and assists the progression from S to early G2 phase. After switching partner, cyclin A-CDK1 takes over from G2 to early M phase. Following the breakdown of cyclin A, cyclin B-CDK1 facilitates M phase transition.

Though cyclin binding is the rate-limiting step for CDK activation, phosphorylation by CDK-activating kinase (CAK) is also needed for CDK activity. Further, CDK activity is repressed via tyrosine phosphorylation by WEE1, which can be removed by the CDC25 phosphatase, thereby de-repressing the CDK. Moreover, ERK can also phosphorylate the cyclin D-CDK4/6 complex during G1 to S phase transition.

On the other hand, there are also several different negative regulators of the cell cycle. First, there are two classes of CDK inhibitory proteins. The first class includes several CDK inhibitors, INK4 proteins (p15, p16, p18 and p19), which specially target CDK4/6, and the CIP1/KIP1 family (p21, p27 and p57), which inhibit cyclin:CDK2 and cyclin:CDK1complexes. For example, p16 can be induced by aberrant oncogenic signals, e.g. RAS (Rayess et al., 2012), after which p16 prevents monomeric CDK4/6 from binding to cyclin D (Hall et al., 1995; Parry et al., 1995; Serrano et al., 1993), and disrupts the pre-formed cyclin D:CDK4/6 complexes (Adachi et al., 1997; Hirai et al., 1995). This prevents pRB from being phosphorylated and inactivated by cyclin D-CDK4/6, thereby maintaining pRB-mediated G1 arrest, as will be explained later. Moreover, p21 can be induced upon physiologic stresses, e.g. DNA damage, in which p21 ceases the activity of already-formed cyclin E:CDK2, cyclin A:CDK2, cyclin A:CDK1 and cyclin B:CDK1 complexes, thereby arresting the cell cycle until DNA is repaired or permanently (senescence) (Harper et al., 1995). Another important factor is the retinoblastoma protein (pRB) (Weinberg, 1995), which regulates the G1-S checkpoint controlling entry into the S phase. pRB is in a hypo-phosphorylated i.e. in an active state in non-cycling cells or during G1 cell cycle arrest. pRB interacts with and represses the transcription factor E2F, which control S-phase genes. During mitotic signaling, cyclin D-CDK4/6 phosphorylates pRB in late G1 phase followed by cyclin E-CDK2, resulting in hyper-phosphorylation of pRB, which inactivates pRB and releases it from E2F. In turn, E2F initiates transcription of S phase-related genes. However, the CDK inhibitor p16, when activated, is capable of maintaining pRB at hypo-phosphorylated state via the repression of CDK4/6, leading to cell cycle arrest at G1 and, if expressed permanently, to cellular senescence (Gius et al., 1999; Takahashi et al., 2007). In addition, pRB also contributes to maintenance of chromatin structure during mitosis, protecting cells from chromosomal instability and aneuploidy, further stressing the importance of pRB as a tumor suppressor (Manning and Dyson, 2011). As a result of this delicate regulatory system, cell cycle is under strict control.

1.1.4 Apoptosis and senescence

When cells are exposed to noxious external agents, or are under oncogenic stress, attacked by the immune system or experience other detrimental signals, cellular fail-safe mechanisms,

such as cellular senescence and cell death, are triggered. Among different types of cell death, apoptosis is a well-studied programmed cell death, in which cells display shrinkage, chromatin condensation and fragmentation into apoptotic bodies. Apoptosis is a complicated and sophisticated process involving an energy-dependent cascade of molecular events. There are generally three classical apoptotic pathways: the extrinsic (death receptor) pathway, the intrinsic (mitochondrial) pathway and granzyme pathway, all of which converge on the same execution pathway eventually (Elmore, 2007).

The extrinsic pathway is triggered by ligands bound to transmembrane receptors mainly of the tumor necrosis factor (TNF) receptor gene superfamily. As an example, during activation of the death receptor (FAS), the FAS ligand (FASL) binds to the receptor to recruit the adapter protein FAS-associated protein with death domain (FADD), and the latter bridges FAS to procaspase 8 to form the death-inducing signaling complex (DISC), leading to the activation (cleavage) of caspase 8. Caspases are cysteine-aspartic proteases that cleave specific protein substrates including other caspases. Activated caspase 8 in turn cleaves and activates caspases 3, 6 and 7, subsequently resulting in execution of the apoptotic process.

The intrinsic pathway is stimulated by stresses, e.g. loss of growth and survival factors, shortage of nutrients or oncogene-induced stress leading to DNA damage. This affects the integrity of the mitochondrial outer membrane, which is controlled by the BCL family apoptosis regulators. For instance, the role of BCL family members BCL2 and BCL-X_L is to keep channels in the mitochondrial membrane closed, while other members, like BAX, BAD, BAK and BID, enable the channels to open. Upon apoptotic signaling, the levels of BAX and BAK increase to exceed that of BCL2 and BCL-X_L on the surface of the mitochondria, leading to the release of cytochrome c - the central actor of intrinsic pathway. Together with APAF1 protein, cytochrome c forms a structure in the cytoplasm called the apoptosome, which activates procaspase 9 to cleave procaspase 3, leading to activation of the executioner caspases 3, 6 and 7, thereby converging with the extrinsic pathway. Many apoptotic stimuli trigger the intrinsic pathway through the activation of p53, which upregulates the expression of BAX, BAK and PUMA, leading to apoptosis. In addition, FAS is a p53 target gene, thereby leading to the sensitization of the cells to the FAS ligand. Thus, p53 can bridge intrinsic and extrinsic apoptotic programs.

A third pathway of apoptosis is triggered by cytotoxic T lymphocytes and natural killer (NK) cells when attacking target cells. In addition to the classic extrinsic pathway to activate death receptors on the target cells, these two types of killer cells can also attach to the target cells and inject granzyme B, a protease that directly cleave and activate procaspase 3 and 8, then converging with the other two pathways, as mentioned above.

Another tumor barrier is cellular senescence, which will be described in more detail in section 1.2.

1.1.5 Tumor immunology

While apoptosis and cellular senescence are important cell-intrinsic barriers against tumorigenesis, the body has also developed a systemic defense system to cope with infection as well as tumor cells.

1.1.5.1 The innate immune system

The innate immune system provides rapid reaction against invasion of foreign organisms, virus-infected cells but also tumor cells. The innate immune system consists of various types of cells, such as macrophages, dendritic cells (DCs), neutrophils and natural killer cells (NKs). Many of them have phagocytic activity and recognize non-self-particles via pattern-recognition receptors, such as toll-like receptor (TLR) family, which in turn, activates cytokine production. For example, after increasing vascular permeability via a number of secretory factors, such as TNF-α, IL-1 and IL-6 (Arango Duque and Descoteaux, 2014), macrophages can migrate to, engulf and destroy the foreign pathogens. Dendritic cells are derived from hematopoietic bone marrow progenitor cells, and initially are immature to prevent adaptive immune cells from attacking host cells. When they encounter foreign entities, they can efficiently phagocytize pathogens and process their antigens, migrate to lymphoid tissue for immune activation and rapidly become mature with secretion of interleukin 12 (IL-12) and type I interferons, leading to activation and proliferation of innate lymphocytes (Steinman and Hemmi, 2006). Natural killer (NK) cells are lymphocytes engaged in cytotoxicity and cytokine secretion, such as interferon-γ (IFN-γ), TNF-α, C-C motif chemokine ligand 2 (CCL2), CCL5 and chemokine (C-X-C) motif ligand 8 (CXCL8), to recruit other immune cells. Besides, via secretion of TNF-α and FASL, NK cells can bind to DR5 and FAS receptors on target cells and induce the latter's cell death. In addition, there are an abundance of activation receptors on NK cells, one of which is NKG2D with a number of stress-related ligands, such as MICA/B and ULBP family proteins, leading to the activation of NK cells to release of lytic granules toward target cells (Vivier et al., 2011; Zamai et al., 1998). Therefore, innate immunity does not require pre-education and offers a rapid array of defenses.

1.1.5.2 The adaptive immune system

Another type of immunity is called adaptive immunity, in which immunological memory is triggered after an initial response to a specific foreign antigen, leading to an enhancement of immune response upon a successive exposure of this antigen. Immunogenic antigens are, however, not limited to foreign antigens, but also can be host-derived, for example tumor antigens caused by gene mutations, so-call neoantigens (Schumacher and Schreiber, 2015). Both T and B lymphocytes play an important role in this process, including helper T cells (Th cells or $CD4^+$ cells), cytotoxic T cells ($CD8^+$ cells or cytotoxic T lymphocytes, CTLs), effector B cells and memory B cells. The activation of T and B cells first requires the processing of antigen by antigen-presenting cells (APCs), mainly macrophages and dendritic

cells. The processed antigenic peptides are loaded onto the specialized antigen-presenting domain of major histocompatibility complex (MHC) class II molecules, in humans called human leukocyte antigens (HLA), and migrate to the surface of APCs, where they are recognized specifically together with HLA class II by $CD4^+$ helper T cells. This leads to activation of the helper T cell, which will start producing cytokines, such as IFN-γ and TNF-α, leading to proliferation and differentiation of cytotoxic T cells. These in turn can kill target cells by triggering apoptotic signals within the target cells via both granzyme pathway and death receptor-mediated extrinsic pathway. A subtype of helper T cells will interact with B cells, resulting in production of effector B cells and memory B cells. The function of effector B is to manufacture corresponding immunoglobulin/antibodies against, which can be secreted into the circulation to defend the foreign invaders and cancer cells. Upon a second exposure of this antigen, memory B cells can rapidly generate effector B cells for antibody production. However, the activity of cytotoxic T cells is restricted by regulatory T cells (T_{reg} cells) that express different inhibitory markers on T_{reg} cells, such cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and lymphocyte activation gene-3 (LAG-3), leading to reduced T-cell proliferation and efficacy. Moreover, transforming growth factor beta (TGF-β) and IL-10 released by T_{reg} suppress proliferation of these T lymphocytes (Corthay, 2009).

Another important component in both innate and adaptive immunity is myeloid-derived suppressor cells (MDSCs) from bone marrow precursors. Its immune suppressive function is accomplished by polarizing macrophages toward a tumor-promoting phenotype producing for instance VEGF and TGF-β that promote tumor metastasis, inhibiting NK-mediated cytotoxicity, repressing the activity of helper and cytotoxic T cells and recruiting T_{reg} cells (Ostrand-Rosenberg and Fenselau, 2018).

1.1.5.3 Tumor immune escape

Because of the efficient surveillance by immune system, cancer cells have developed different strategies of immunoevasion. Cancer cells often repress the expression of MHC class I, thereby avoiding tumor antigen presentation to cytotoxic T cells. Moreover, they can also release soluble FASL or immunosuppressive cytokines (TGF-β and IL-10) to sensitize cytotoxic T cells or DCs to apoptosis (Neuzillet et al., 2015; Peter et al., 2015). In addition, tumor cells can also activate T_{reg} to escape the killing by variety of T lymphocytes. Further, in order to inactivate T cells, similar to T_{reg} cells, cancer cells can stimulate immune checkpoint pathways by increasing expression of the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death-ligand 1 (PD-L1), to dampen the function and proliferation of cytotoxic T cells. By these ways, cancer cells try to promote 'immune escape'.

1.1.6 Malignant melanoma and neuroblastoma

I will next briefly introduce two types of cancer, melanoma and neuroblastoma, which are particularly related to my research, and how they are treated.

1.1.6.1 Malignant melanoma

Due to its aggressiveness at the advanced stage, malignant melanoma is considered as one of the most deadly forms of cancer, and its incidence is increasing worldwide. Sunlight exposure is one of main pathogenetic contributors. Cutaneous melanoma can be broadly classified in two categories: chronically sun damaged (CSD) and non-CSD melanomas, which also have difference in anatomical site of origin, mutation burden and types of oncogenic alteration. In CSD melanoma, due to long-term ultraviolet (UV) exposure, melanoma typically arises from the head, the neck and the dorsal surfaces of the distal extremities of older individuals, associated with mutations on NF1, NRAS, $BRAF^{nonV600E}$ or KIT. However, non-CSD melanoma originates from areas with less UV exposure, such as trunk, which is predominantly related to $BRAF^{V600E}$ mutations at melanocytic nevi (Shain and Bastian, 2016). In general, $BRAF^{V600E}$ occurs in 50% of melanoma and NRAS mutation accounts for 15-20% of cases, leading to aberrant signaling in MAPK pathway. Upon additional hits in driver genes such as PTEN loss and p53 mutation, these benign symmetrical nevi take an irregular shape and converts to a malignant stage. Excision surgery is always the first option to treat melanoma. But if the tumor is unresectable or have metastasized to other part of body, other therapies have to be applied.

1.1.6.1.1 Traditional radiation and chemotherapy

Chemotherapy includes the alkylating agents dacarbazine and its oral version-temozolomide, cisplatin, fotemustine and etc. Radiation therapy, utilizing high-energy X-ray or other particles, is another option to suppress tumor progression and as palliative therapy. However, both these treatment cause side effects and are not curative at advanced stages of disease.

1.1.6.1.2 Targeted therapy

Targeted therapy is designed for personalized therapy to specifically target cancer cells for each patient, based on the presence of critical driver mutations. Vemurafenib was the first drug targeting melanoma with $BRAF^{V600E}$ mutation, and has been a great success in treating these patients (Sosman et al., 2012). However, the therapeutic effect of vemurafenib is usually only transient due to development of drug resistance, which limits the application of vemurafenib in the clinic (Wagle et al., 2011). Inhibitors against MEK, a downstream effector of BRAF, is another new option, but also here tumors become resistant (Falchook et al., 2012). Many resistance mechanisms have been suggested, such as reestablishment of the MAPK pathway, activation of the PI3K/AKT pathway or upregulation of RTKs (Das Thakur et al., 2013; Sullivan and Flaherty, 2013; Wagle et al., 2014; Welsh et al., 2016). In order to overcome resistance, combination therapy using trametinib (MEK inhibitor) together with dabrafenib ($BRAF^{V600E}$ inhibitor), or vemurafenib ($BRAF^{V600E}$ inhibitor) combined with cobimetinib (MEK inhibitor) have attracted attention, and have been approved by the Food and Drug Administration (FDA) for unresectable or metastatic melanoma exclusively, while many other combinations are undergoing clinical trials.

1.1.6.1.3 Immunotherapy

Another recent option for melanoma treatment is immunotherapy, with the aim to invoke the patients' natural defenses against cancer. Melanoma is considered as one of the most immunogenic types of cancer based on the following: many genes are mutated due to UV exposure, which is the main trigger of melanoma, causing the generation of a number of neoantigens. For example, researchers have identified several melanoma-specific antigens, such as melanoma antigen-encoding (MAGE) and NY-ESO-1. Moreover, melanoma patients acquire large amounts of melanoma-specific antibodies. In addition, metastatic melanoma is responsive to immune-stimulatory agents, including interferons and interleukin 2. Another hint is that patients with spontaneous regression have developed vitiligo (Jacobs et al., 2012) and the frequent observation of tumor infiltrating lymphocytes (TILs) into the tumor area, which is positively correlated with prognosis in many studies (Oble et al., 2009). Thus, immunotherapy for melanoma has drawn much attention and become the first-line therapy for melanoma with non-BRAF mutation.

As mentioned above, CTLA-4 and PD-1 are two molecules responsible for the self-inhibitory pathways on T cells, so it is tempting to think that inhibition of either of these two molecules might enhance the patients' immune response. FDA has approved three drugs, including Ipilimumab (anti-CTLA4 antibody), opdivo and pembrolizumab (anti-PD-1 antibodies), for melanoma treatment. In a phase 3 trial in resected stage III or IV melanoma, overall survival at 12 months in opdivo group is 70.5%, versus 60.8% in ipilimumab group (Weber et al., 2017); while in another phase 3 trial in advanced melanoma, overall survival at 12 months in pembrolizumab group is 74.1% versus 68.4% in ipilimumab group (Schachter et al., 2017). However, the problem of resistance is inevitable in immunotherapy as well in advanced melanoma (Zaretsky et al., 2016). Further, it has been difficult to identify clinically reliable biomarkers for selection of patients that would benefit of immunotherapy. PD-L1 has been suggested as a promising biomarker for immunotherapy, but in several pivotal trials, PD-L1 negative patients also responded to treatment to a moderate or even better extent, implying the involvement of other unknown mechanisms for sensitivity to this treatment (Robert et al., 2015; Weber et al., 2015).

1.1.6.2 Treatment on neuroblastoma

Neuroblastoma, which is a childhood tumor derived from the neural crest during development, is the most common extracranial solid tumor for children, accounting for 6-10% of all childhood cancers. The tumor is due to uncontrolled proliferation of immature neuroblasts of the peripheral sympathetic nervous system (Brodeur, 2003). The cancer can occur anywhere along the peripheral nervous system and in about 50% of the cases it starts in the adrenal gland in the abdomen, causing a swollen belly and constipation. *MYCN* amplification is strongly correlated to the severity of the disease (Seeger et al., 1985). It has also been reported that mutations in the *ALK* and *PHOX2B* genes, as well as deletion of certain loci of chromosome 1 and chromosome 11, are associated with sporadic and familial

neuroblastoma (Molenaar et al., 2012). Low-risk patients, that often undergo spontaneous regression, do not receive therapy, but high-risk cases with high refractory rate and poor prognosis need more aggressive treatment. A variety of treatment options are available for high-risk cases, including several chemotherapeutic drugs, e.g. cisplatin and etoposide, followed by surgery, radiotherapy and maintenance therapy by cis-retinoic acid. However, the overall survival for high-risk group, most resistant to treatment, is 50% (Johnsen et al., 2018). Novel treatment strategies are therefore urgently needed for these patients.

1.2 CELLULAR SENESCENCE

The second part of my thesis is about cellular senescence, particularly premature senescence in cancer biology.

Senescence is characterized by irreversible cell cycle arrest, irrespective of the presence of growth factors. Unlike cells undergoing apoptosis or quiescence, senescent cells have a high metabolically activity, possess increased β-D-galactosidase activity, are phenotypically enlarged, have remodeled their high order chromatin structure and exhibit a senescence-associated secretory phenotype (SASP), as summarized in Fig. 1.

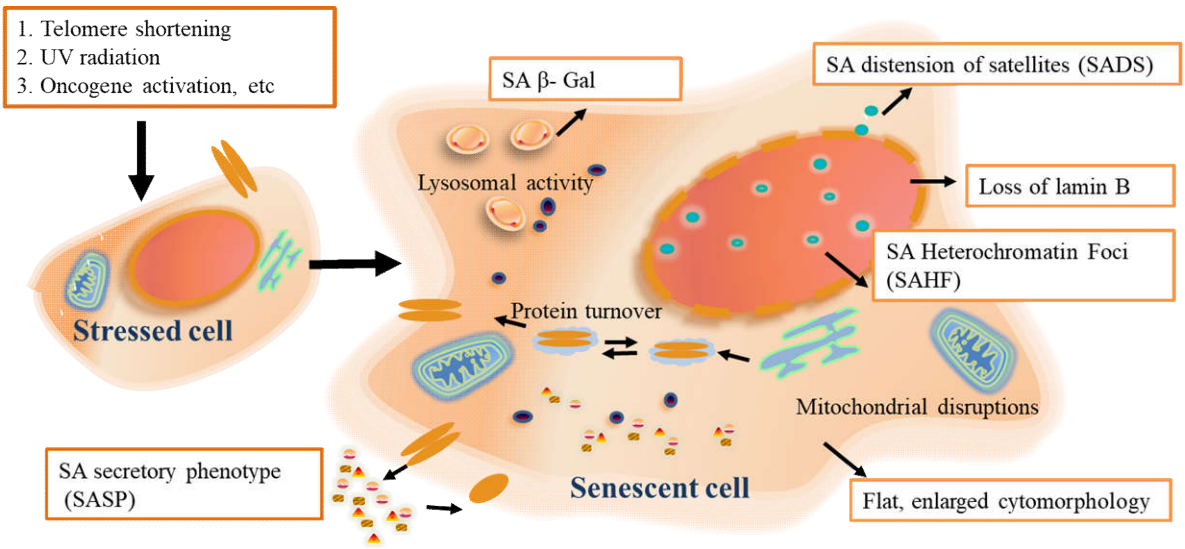

Fig. 1. Causes and characterization of cellular senescence.

Cellular senescence is, together with apoptosis, a main barrier against tumorigenesis, but is also involved in normal physiological cellular processes. For example, senescence occurs during mammalian embryonic development, and is engaged in tissue remodeling (Munoz-Espin et al., 2013). Senescence also plays a role in wound healing and tissue regeneration (Chiche et al., 2017; Demaria et al., 2014). Senescent cells secret SASP factors consisting of proinflammatory factors, growth factors and protease, etc, to modify microenvironment and recruit immune cells. Further, it is implicated in aging and age-related pathologies resulting in accumulation of senescent cells, thereby compromising tissue function and depletion stem

cell pools (Childs et al., 2015).

1.2.1 Replicative senescence and premature senescence

Telomere erosion is one trigger of senescence, which is called ‘replicative senescence’ and associated with the cellular aging process.

Telomeres, which are composed of the 5’-TTAGGG-3’ replicative hexanucleotide sequence, are located at the ends of chromosomes and are protected by shelterin complex protein and RNA transcribed from telomeres. During DNA replication of the lagging strand, DNA polymerase can only continue the duplication in the presence of Okazaki fragments, which requires RNA primers to start replication. This causes the so-called end replication problem since the ends of the chromosomes cannot be completely duplicated. This leads to successive shortening of the telomeres in each cell cycle. If telomeres get too short, the structure of the telomere/shelterin complex is compromised, leading to uncapping of the telomeres. The unprotected free DNA ends will induce DDR and then be recognized by DNA repair system, such as non-homologous end joining (NHEJ), homology directed repair (HDR), which can lead to end-to-end fusion of telomeres from different chromosomes (Sfeir and de Lange, 2012). If two chromosomes are fused together, there will be two or more centromeres, and the latter will be pulled to opposite directions by the mitotic spindle afterwards, subsequently leading to ripping chromosomes apart at some random sites. Initial telomere erosion and chromosome breaks induce further DDR, which in turn triggers replicative senescence, also called mortality stage 1 (M1) during cellular aging. However, if cells bypass M1 due to the defect of cell-cycle checkpoint mechanism (p53/p21 or p16/pRB pathway), end-to-end fusion becomes more severe and breakage fusion-bridge accelerates, which eventually results in crisis (M2 stage). However, in rare cases, some M2 cells will survive with upregulation of telomerase and possess indefinite cell proliferation, which is called immortalization (Fridman and Tainsky, 2008; Shay and Wright, 2005). Cancer cells that have escaped replicative senescence are immortal, capable of growing indefinitely.

On the other hand, senescence can also be triggered by variety types of acute stresses, which is called premature senescence and is the focus of this thesis. For example, aberrant activation of oncogenes like RAS can induce senescence, in this case so called oncogene-induced senescence (OIS). Besides OIS, premature senescence can also be stimulated by other types of stresses, e.g. UV irradiation, hypoxia or toxins etc.

The main players in senescence induction and maintenance are two tumor suppressor pathways - the p53 pathway and pRB pathway (Collado and Serrano, 2010; Larsson, 2011). p53 functions as a tetrameric transcription factor that is highly unstable with a half-life of 20 minutes in normal cells. It is degraded predominantly by the E3 ubiquitin ligase mouse double minute 2 homolog (MDM2). However, p53 protein level is increased upon many types of stresses post-translational modification- mainly phosphorylation (Horn and Vousden, 2007). For example, uncontrolled RAS activity causes DNA replication stress, leading to

double stranded DNA breaks. This leads to recruitment of the DDR sensor ataxia-telangiectasia-mutated (ATM), followed by the activation of checkpoint kinase 2 (CHK2). CHK2 in turn phosphorylates p53 at Serine 20, which is part of MDM2 binding site, resulting in displacement of MDM2 and p53 stabilization. Moreover, ATM/ ataxia telangiectasia and RAD3-related (ATR) can also phosphorylate MDM2 and suppress MDM2-mediated p53 degradation. After p53 activation, its downstream effector p21 is induced to arrest the cell cycle by inhibiting CDK2 (Aird et al., 2013; Di Micco et al., 2006). Moreover, pRB is important for senescence induction, for example through repression of a subset of E2F target genes linked to DNA replication (Chicas et al., 2010). In addition, p16 can be induced by oncogenic RAS, resulting in inhibition of CDK4/6 thereby preventing pRB phosphorylation and inactivation, reinforcing pRB-mediated suppression of E2F activity and subsequently cellular senescence (Mason et al., 2004). Therefore, p16/pRB is considered as a crucial pathway in senescence induction via both pRB-mediated G1/S control and p16-triggered CDK block. Recently, it has been reported that upon oncogenic stress, ribosome biogenesis defects occur after which the ribosomal protein S14 (RPS14) accumulates in nucleus and suppresses the activity of cyclin D/CDK4 complex, leading to preservation of active state of hypo-phosphorylated pRB, which trigger senescence (Lessard et al., 2018). Further, pRB also has functions independent of E2F repression. One is through promoting degradation of S-phase kinase associated protein 2 (SKP2) by recruiting it to its ubiquitin ligase CDH1. In this way, pRB induces accumulation of one of substrates of the ubiquitin ligase SKP2, p27, which in turn inhibits CDK2 and CDK1 and thereby reinforce senescence (Binne et al., 2007; Wang et al., 2010).

In addition, it has been reported that not only highly proliferating tumor cells, but also slowly proliferating cancer stem cells can undergo senescence (Kuilman et al., 2010). Therefore, cellular senescence, particularly premature senescence, functions as a blockage of tumorigenesis. Induction of premature senescence has therefore been suggested as anti-tumor therapy (pro-senescence therapy). In the following, I will go through the senescence concept in more detail.

1.2.2 Senescence and autophagy

β-D-galactosidase was the first senescence marker to distinguish senescent cells from terminally differentiated cells or quiescent cells, and is widely used in senescence research (Dimri et al., 1995). Physiologically, this enzyme is activated as a eukaryotic hydrolase at pH4.0-4.5 in the lysosome (D'Azzo et al., 1982). However, in senescent cells, this enzyme is also active at pH 6 due to both increased amount and activity (Kurz et al., 2000). This can be measured by the addition of the artificial substrate X-GAL. However, SA-β-GAL activity is only a marker for senescence, its role in the senescence process seems dispensable (Lee et al., 2006). Later on, researchers also found other components in the lysosome that can be used as senescence markers, including α-L-fucosidase and Sudan-Black-B specific staining of

lipofuscin (Georgakopoulou et al., 2013; Hildebrand et al., 2013). However, it remains unclear how the lysosome is functionally intertwined with senescence.

Besides senescence, autophagy is another lysosome-linked cellular process. Under both physiological and stressful conditions, autophagy serves as an evolutionary ancient process to supply and maintain nutrients and energy by degrading as well as recycling damaged proteins and organelles. This is mainly regulated by autophagy-related *ATG* genes and the mTOR complex. Conceptually, both autophagy and senescence play important intrinsic roles in protecting cells from a variety of shared stimuli, including telomere shortening, DNA damage and oncogenic stress. Thus, it is tempting to assume that there is a crosstalk between these two cellular processes. Indeed, the role of autophagy has been explored in oncogene-induced senescence; in a HRAS-induced senescence model, Young et al. discovered that inhibition of autophagy via PI3K-mTOR pathway can delay senescence induction and block the protein synthesis process of SASP, indication a positive relation between senescence and autophagy (Young et al., 2009). This relationship was also supported by other studies (Huang et al., 2014; Liu et al., 2014; Takasaka et al., 2014). Interestingly, it also has been documented an autophagic degradation of the nuclear lamina component lamin B, in particular during oncogene-induced senescence but not during starvation (Dou et al., 2016; Dou et al., 2015). However, other independent studies report a negative correlation between senescence and autophagy. For example, knockdown of ATG5 in mouse fibroblasts led to enhanced RAS-induced senescence induction (Wang et al., 2012). One explanation of this discrepancy might be differential levels or types of autophagic activities in different cells. For instance, severe oxidative stress that exceeds the autophagic capacity can suppress autophagy and trigger senescence (Han et al., 2016; Tai et al., 2017). It can also be speculated that certain processes or components linked to autophagy are also required for senescence, or that there is a certain balance between two activities, implying a sophisticated relationship between senescence and autophagy. However, recent studies also question the role of autophagy in senescence. One study showed that autophagy is not involved in telomere dysfunction-induced senescence, and yet another study suggested that the two processes are not linked in glioma cells (Filippi-Chiela et al., 2015; Mar et al., 2015). Overall, the relationship between senescence and autophagy remains unclear and needs to be investigated further.

1.2.3 Senescence-associated chromatin remodeling

Another characteristic of senescent cells is chromatin remodeling. During senescence, cells increase not only their cellular size, but also the nuclear size, which is linked to chromatin reorganization. This was first described by Narita and his co-workers, where they discovered the formation of senescence-associated heterochromatin foci (SAHF) (Narita et al., 2003). SAHF are distinct punctuate DAPI-dense foci, enriched in repressive histone marks, e.g. histone hypoacetylation, macroH2A, H3K9me3 and histone H3 lysine 27 trimethylation (H3K27me3). pRB is required for the formation of SAHF, and many pRB-regulated genes, e.g. cell cycle-related E2F target genes, are localized within the SAHFs. In turn, the

suppression of such genes in SAHFs promotes and stabilizes senescence. Furthermore, two repressive marks, H3K9me3 and H3K27me3, within the SAHFs are localized in non-overlapping layers, surrounded by pericentromeric constitutive heterochromatin, leading to segregation of heterochromatin and euchromatin in these structures (Chandra et al., 2012). Unexpectedly, genome-wide occupancy profile by chromatin immunoprecipitation (ChIP) sequencing found no significant changes of these two repressive marks in the global chromatin landscape, except in certain specific genic regions, such as within the *CDKN2A (p16)* and *CCNA2 (cyclin A2)* loci. Moreover, depletion of these two marks failed to disrupt SAHF formation and vice versa, indicating that high-order heterochromatin formation is separable from epigenetic remodeling.

The formation of SAHF has been further dissected by Peter Adams's group. They found, firstly, that heterochromatin protein HP1γ and histone repressor A (HIRA) are recruited to promyelocytic leukemia (PML) nuclear bodies prior to SAHF formation. After binding of antisilencing function 1a (ASF1a) to HIRA and histone H3, this complex is capable of condensing individual chromosomes. SAHF is formed following integration of macroH2A or HP1γ (Zhang et al., 2007; Zhang et al., 2005). This is completed with further deposition of high-mobility group A (HMGA) proteins (Narita et al., 2006). However, HP1 proteins seemed to be dispensable for SAHF formation (Zhang et al., 2007). SAHF formation therefore remains elusive.

Further studies also revealed that SAHF formation is a two-step event in 'deep senescence' (see further discussion about "deep senescence" below in section 1.2.5). First there is a loss of local interactions in certain regions of heterochromatin from the nuclear lamina, after which these regions form a senescence-specific spatial clustering of heterochromatin as a potential core of SAHF (Chandra et al., 2015).

Due to the silencing capacity of SAHFs, it is considered to contribute to cell cycle arrest via repression proliferation-related genes (Narita et al., 2003; Zhang et al., 2007). An alternative interpretation is that SAHFs restrain the DNA damage response to avoid apoptosis, which would prolong the viability of senescent cells (Di Micco et al., 2011). However, due to observations that SAHF formation is not universal during senescence but cell context- and stimuli-dependent, SAHF cannot serve as a general senescence biomarker (Di Micco et al., 2011; Kosar et al., 2011).

Besides the distinct SAHF patterns, there are other alterations occurring in nucleus during senescence. As shown by Criscione et al. (Criscione et al., 2016), in general, long-range interactions are relatively lost and short-range gained within chromatin. Chromatin compaction occurs in the chromatin arms, leading to reduced size and poor accessibility of transcription machinery to these regions, resulting in gene suppression, while the centromeres expand in volume. The decompaction of centromemic DNA is also consistent with an earlier study from Swanson and colleagues (Swanson et al., 2013). They showed that at the early stage of senescence, DNA satellites in human and mouse centromeres are unraveled, which is termed as senescence-associated distension of satellites (SADS). This phenomenon appears

in human and murine cells both *in vitro* and *in vivo*, but it is not caused by nuclear enlargement and does not rely on canonical histone marks-mediated chromatin condensation. Further, in contrast to SAHFs it is stimuli-independent. The potential function of SADS could be to enhance the persistence of senescent state via blocking cell cycle, although this remains to be clarified. The enlargement of nuclear size observed during senescence occurs in parallel with an increase of nuclear protein content (De Cecco et al., 2011).

Another important nuclear change during senescence occurs in nuclear lamins, which constitute the main structural component of nuclear lamina and are localized in the inner part of the nuclear envelope. There are generally two types of lamins, A-type (lamin A/C) and B-type (lamin B). The former is mainly expressed in fully differentiated cells while the latter one is present ubiquitously in the lamina (Hutchison, 2002). In particular, it has been shown that rather than caspase-mediated degradation, the lamin B1 mRNA level decreases during senescence (Freund et al., 2012). The decrease in lamin B is suggested to contribute to the rearrangement of heterochromatin and SAHF formation. However, it remains controversial whether lamin B plays a causal role in senescence since lamin B knockdown merely slowed down proliferation rather than induced senescence in human fibroblasts (Dreesen et al., 2013).

Moreover, chromatin budding also appears in late senescent cells, due to the loss of lamin B1 and subsequently the integrity of nuclear envelope. These buddings fragments are expelled from nucleus into cytoplasm, are positive for γ-H2AX and H3K27me3, and form so-called cytoplasmic chromatin fragments (CCFs). The latter is targeted by autophagy machinery (Ivanov et al., 2013).

1.2.4 Senescence and mitochondria

One of important organelle, mitochondria, has also been reported to associate with senescence. In contrast to quiescent or apoptotic cells, senescent cells are metabolic active, characterized by increased oxygen consumption, mitochondrial potential, energy production and reactive oxygen species (ROS). Besides these senescence-associated features, emerging evidence has shown that mitochondrial dysfunction contributes to senescence induction. Back to the 50s, Harman et al. already proposed that mitochondrial free radicals contribute to aging (Harman, 1956). Nowadays, it is widely accepted that ROS is a senescence inducer. For example, exogenous hydrogen peroxide (H_2O_2) can generate endogenous ROS, including superoxide and hydroxyl radicals, leading to nuclear DNA damage and DDR-induced activation of the p53/p21 pathway, subsequently leading to cell cycle arrest (Duan et al., 2005; Zdanov et al., 2006). This increased steady-state level of ROS is considered as a positive feedback loop to maintain senescence, but other studies indicated that ROS production is dispensable after initiation of ROS-induced senescence (Passos et al., 2010). Apart from extra ROS generation, senescence is also linked to impaired mitochondrial dynamics, i.e. fission and fusion of mitochondria. For example, upon depletion of fission 1 (FIS1) - a mediator of mitochondrial fission – the fission process is compromised and the mitochondria remain elongated, which resulted in senescence (Lee et al., 2007). Additionally, mitochondrial fusion

is implied to play a protective role when cell are stressed by stimulation of ATP synthase activity and prevention of mitochondrial depolarization, but the actual contribution is still under investigation (Gomes et al., 2011). Further, the electron transport chain (ETC) in mitochondria has also been implicated in senescence induction. For example, either pharmacological inhibition of ETC or genetic knockdown of the electron transporter can lead to senescence (Moiseeva et al., 2009; Yaguchi et al., 2007). Moreover, ETC-generated ATP also plays a role in senescence regulation. ATP depletion or exogenous AMP addition leads to disequilibrium of mitochondrial bioenergetics and activation of AMP-activated protein kinase (AMPK), followed by cell cycle arrest and senescence (Mihaylova and Shaw, 2011; Zwerschke et al., 2003). In addition, suppression of mitochondrial sirtuins (SIRTs) decreases the ratio of NAD^+/NADH, leading to the activation of AMPK/p53-induced senescence, which accompanied with a distinct secretory phenotype and can be rescued by addition of pyruvate (Wiley et al., 2016). Therefore, mitochondria homeostasis is indeed closely linked to cellular senescence via the different factors mentioned above.

1.2.5 Multi-step senescence model

Rather than a static state, senescence is increasingly recognized to be a dynamic multi-step process (Baker and Sedivy, 2013; van Deursen, 2014). As summarized by Baker et al, cells are first temporally arrested in response to senescence inducers, so-called initiation of senescence response. In contrast to the conventional definition of cellular senescence as irreversible cell cycle arrest, this dynamic model provides more options for cells besides senescence, e.g. quiescence, terminal differentiation, cell death or re-entering cell cycle. In order to complete the transition from temporal to permanent senescence, more factors need to participate, such as persistent expression of tumor suppressor p53, or continuous p21/p16-mediated inhibition of CDK-cyclin activity, or durable DNA damage response and so on. Without these events senescence cannot be reinforced or maintained. However, the accurate definition of ‘full’ senescence remains incomplete. It is still unclear what changes need to occur during maturation of senescence, e.g. the downregulation of lamin B1, the formation of SAHF or the decondensation of peri/centromric satellite heterochromatin, which contributes to massive alteration of transcriptome and secretome. Further, this ‘deep’ or ‘late’ senescence is also poorly defined due to lack of reliable biomarkers. As suggested, the striking increase of the transcription of transposable elements, e.g. members of L1, ALU and SVA, could be associated with ‘deep’ senescence (De Cecco et al., 2013; Wang et al., 2011). This leads to heterochromatin opening and loss of nuclear integrity, in another word, substantial alteration of genomic and epigenetic landscape, which could be one of the requirements for deep senescence.

1.2.6 Senescence-associated secretory phenotype (SASP)

The senescence-associated secretory phenotype (SASP) is one of the important characteristics to distinguish senescent cells from other non-proliferation cells, e.g. terminally differentiated

or quiescent cells. SASP has a heterogeneous profile, including inflammatory cytokines, chemokines, growth factors/regulators, proteases and insoluble proteins/extracellular matrix (ECM) components, which is cell context- and stimuli-dependent (Coppe et al., 2010; Munoz-Espin and Serrano, 2014; Sharpless and Sherr, 2015).

Transcriptionally, SASP expression is mainly mediated by nuclear factor-κB (NF-κB) and CCAAT/enhancer binding protein-β (C/EBPβ) transcription factors, in response to different types of senescence stimulatory pathways, such as the RAF/MEK/ERK, p38MAPK and mTOR pathways (Chien et al., 2011; Freund et al., 2011; Kuilman et al., 2008). Apart from SASP transcription, mTOR signaling regulates SASP at translational level via stabilization of mitogen-activated protein kinase-activated protein kinase 2 (MK2), which in turn, avoid degradation of many SASP transcripts (Herranz et al., 2015). However, it remains unclear about the sophisticated mechanisms of SASP regulation.

Due to the diverse composition of SASP (Hernandez-Segura et al., 2017), it is still under debate whether SASP exerts tumor suppressive or pro-tumorigenic effects. There are many SASP factors are associated with tumor suppression. For example, IL-6 is commonly involved in cell proliferation, survival and differentiation. In the senescent setting, it has been suggested to both reinforce senescence in an autonomous fashion and also trigger neighboring cells senescent in a paracrine manner (Kojima et al., 2012; Kuilman et al., 2008; Wan et al., 2014). In addition, CXC chemokine receptor 2 (CXCR2) is a member of the G-protein-coupled receptor family, and it has many ligands, such as CXCL1and CXCL8, leading to activation of G-protein-mediated second messenger system. This receptor is associated with neutrophil migration to inflammation site (Futosi et al., 2013). CXCR2 has been reported to reinforce senescence in MEK-induced senescence *in vitro* via DDR-dependent p53 activation (Acosta et al., 2008). Moreover, chemokine CCL5 is involved in recruitment of leukocytes into inflammatory sites, and proliferation and activation of NK cells (Maghazachi et al., 1996). Vilgelm et al. revealed the important role of CCL5 in tumor regression in two different settings of therapy-induced senescence (TIS). Upon combination treatment of aurora kinase A (AURKA) inhibitor and MDM2 antagonist, senescent melanoma secreted CCL5, which in turn, triggered immune surveillance by recruitment of NK, DC and T cells, leading to tumor clearance (Vilgelm and Richmond, 2015). In another model, CDK4/6- or AURKA-inhibitor treatment promoted TIS-induced CCL5, which resulted in recruitment of tumor-infiltrating leukocytes (TILs) and enhanced immunotherapy efficacy (Vilgelm et al., 2016). Further, the chemokine CCL2 has been reported to recruit $CCR2^{+}$ immature myeloid cells (iMC) and to trigger their differentiation into macrophages, which remove senescent cells (Eggert et al., 2016). Furthermore, other proinflammatory cytokines, e.g. IL-1 and TGF-β, are capable of activating ROS-mediated DDR and p15/p21induction, leading to export of senescence in a paracrine manner (Hubackova et al., 2012) (Acosta et al., 2008). IL-1α is reported to be the master regulator upstream of IL-6 and IL-8 via activation of inflammasome, and blockade of either IL-1 or inflammasome affected paracrine senescence, indicating the importance of IL-1 in the regulation of SASP and senescence (Acosta et al., 2008). Interestingly, $Gr\text{-}1^{+}$ myeloid-derived suppressor cells were

shown to antagonize IL-1α-signaling during docetaxel-induced senescence in a PTEN null prostate tumor model through secretion of IL-1α receptor antagonist (IL-1RA) (Di Mitri et al., 2014). TNFα is another SASP-related factor that can induce senescence by activation of p38-MAPK and NF-κB pathways (Wajant et al., 2003) and recruit NK cells to target senescent tumor cells *in vivo* (Beyne-Rauzy et al., 2004; Ruscetti et al., 2018).

However, SASP can be a double-edged sword since it has also been shown to promote tumorigenesis under certain conditions. Indeed, many of SASP factors are pro-inflammatory factors with paracrine function, which may cause detrimental effects in the long run, such as chronic inflammation, linked to degenerative diseases and cancer (de Visser et al., 2006; Glass et al., 2010; Grivennikov et al., 2010). Therefore, these aspects are considered the dark side of SASP (Coppe et al., 2010; Georgilis et al., 2018). For example, normal senescent fibroblasts in the microenvironment can induce epithelial cell proliferation and tumorigenesis (Krtolica et al., 2001); promote angiogenesis (Coppe et al., 2006); accelerate epithelial to mesenchymal transition leading to tumor cell invasion (Coppe et al., 2008). Moreover, due to the secretion of SASP, stromal cells may function as cancer-associated fibroblasts (CAFs) to communicate and facilitate tumor progression. For instance, senescent stromal cells can recruit suppressive myeloid cells (MDSCs) via SASP to antagonize anti-tumor-T cell function, leading to tumorigenesis (Ruhland et al., 2016). Additionally, an immunosuppressive microenvironment is generated by SASP in certain cases, which can undermine chemotherapy efficacy (Toso et al., 2014). In addition, SASP composition and function is dynamic and cell context-dependent, and therefore even for the same factor can play either a positive or negative role in tumorigenesis depending on the context. One example is CXCR2, which reinforces senescence in KRAS-induced senescent fibroblasts as a tumor suppressor (Acosta et al., 2008). However, once premalignant cells escape senescence and develop cancer, these cells downregulate CXCR2 expression and recruit MDSCs with high oncogenic CXCR2 level, leading to tumor metastasis, like in pancreatic ductal adenocarcinoma (PDAC) with KRAS mutation (Steele et al., 2016). As mention above, CCL2 secreted by senescent pre-malignant hepatocytes recruit $CCR2^+$ immature myeloid cells for clearance, but if these cells are mixed with malignant cells, the recruitment of $CCR2^+$ immature myeloid cells will promote tumor growth (Eggert et al., 2016).

Taken together, if and when SASP is good or bad for tumorigenesis is controversial and still under debate.

1.2.7 Pro-senescence therapy

The existence of senescence in tumors *in vivo* was first presented in four independent publications in 2005, in which senescent cells appeared in benign tumors but not in invasive cancer samples from prostate cancer, melanocytic nevi and indolent lymphomas (Braig et al., 2005; Chen et al., 2005; Collado et al., 2005; Michaloglou et al., 2005). Prior to this it was debated whether senescence was an *in vitro* phenomenon, but this undoubtedly confirmed that senescence occurs *in vivo* and serves as a barrier against tumorigenesis. With increasing

evidence of senescence-mediated tumor suppression, the concept of pro-senescence therapy, also called therapy-induced senescence, has emerged a novel treatment strategy for various tumors, especially in tumors resistant to apoptosis-inducing therapy (Acosta and Gil, 2012; Larsson, 2011; Nardella et al., 2011).

The tumor suppressive potential of pro-senescence therapy does not only rely on the irreversible cell cycle arrested feature of senescence, but also depends on SASP-triggered immune surveillance. The first evidence of the latter was that, after reactivation of RNAi-regulated endogenous p53 in the mosaic mouse model of liver carcinoma, senescence was induced coupled with SASP consisting of different inflammatory cytokines that recruit macrophages, neutrophils and natural killer (NK) cells for clearance of senescent tumor cells (Xue et al., 2007). The recruitment of NK cells in this system was shown to be mediated by secretion of the SASP factor CCL2 (Iannello et al., 2013). Further, using a KRAS-driven lung tumor mouse model, a recent publication showed that upon combination treatment with CDK4/6 and MEK inhibitors, induction of senescence in the lung cancer cells led to recruitment of NK cells and tumor clearance via the SASP components TNFα and intercellular adhesion molecules-1 (ICAM-1) (Ruscetti et al., 2018). M1-like macrophages also play a positive role in tumor clearance upon p53 inhibition-triggered senescence in liver cancer (Lujambio et al., 2013).

In addition to innate immune response, a study by Rakhra et al demonstrated that $CD4^+$ T-cells were required for the initiation of immune clearance in MYC-induced T cell acute lymphoblastic lymphoma or BCR-ABL-mediated pro-B cell leukemia mouse model (Rakhra et al., 2010). As mentioned above, triggering of senescence in melanoma cells upon dual inhibition of AURKA and MDM2 induced secretion of CCL5, leading to tumor clearance by activation of several immune cell types, including NK cells, DC, macrophages and T cells (Vilgelm and Richmond, 2015).

Furthermore, pro-senescence therapy can also enhance the efficacy of immunotherapy. For example, CDK4/6 inhibition-induced senescence was reported to enhance anti-tumor immunity not only by suppressing the T_{reg} population, but also by promoting cytotoxic T cell activity, which improved anti-PD-L1 immunotherapy (Goel et al., 2017). This also explains the enhanced antitumor activity with dual inhibition of BRAF and MEK combined with immunotherapy in melanoma (Hu-Lieskovan et al., 2015). Further, the potency of T cell-activating therapy was improved together with AURKA-induced senescence therapy (Vilgelm et al., 2016). Thus, pro-senescence therapy indeed has the anti-tumor potential.

However, as mentioned in section 1.2.6, SASP may create an immunosuppressive microenvironment, driving tumorigenesis and undermines chemotherapy efficacy (Ruhland et al., 2016; Toso et al., 2014). Thus, pro-senescence therapy should ideally be followed up by complementary treatment to eliminate senescent tumor cells. One theoretically conceivable therapeutic strategy is to provoke or reinforce immune response via the modulation or reprogramming of SASP. For example, in a mouse model of senescent PTEN-null prostate tumor cells, inhibition of JAK2/STAT3 (Janus kinase/signal transducers and activators of

transcription) can reprograme SASP, leading to a switch of secretion profile from immunosuppressive to immunostimulatory, resulting in further enhancement of chemotherapy efficacy, which supports the feasibility of pro-senescence together with immunotherapy (Toso et al., 2014).

Although therapies based on elimination of senescent cells by immune system is an attractive approach, it is also challenging due to cell context- and stimuli-dependent heterogeneity of SASP and its interaction with the innate and adaptive immune system.

Another option to eliminate senescent cells is via senolysis, which is an issue that has been raised recently to selectively kill senescent cells as adjuvant therapy to pro-senescence therapy. Actually, already back to 1995, Eugenia Wang proposed that senescent cells are resistant to apoptosis due to upregulation of BCL-2 (Wang, 1995), which inspired Kirkland's group to try to target senescent cells via interfering with their anti-apoptotic, pro-survival mechanisms. After comparing the transcriptome of proliferative and senescent primary cells, they revealed the related pathways including BCL-2/BCL-X_L, PI3K/AKT, p53/p21/serpines and different dependence receptors/tyrosine kinases (Zhu et al., 2015). The tyrosine kinase inhibitor dasatinib and PI3K inhibitor quercetin were identified as the first senolytic drugs, followed with inhibitors in BCL-2/BCL-X_L pathway, such as navitoclax, A1331852 and A1155463 (Chang et al., 2016; Zhu et al., 2017; Zhu et al., 2016b). Related to quercetin, fisetin, has been identified as another senolytic drug with relatively low cytotoxicity (Yousefzadeh et al., 2018).

In addition to apoptosis, there are also other synthetic lethal approaches targeting senescent cells. Since they are linked to high metabolic activity, senescent cells are susceptible to inhibition of specific metabolic factors, such as glucose transporters, glycolysis, energy sensor AMPK and pyruvate dehydrogenase kinase 1 (PDK1) (Dorr et al., 2013; Kaplon et al., 2013; Wiley and Campisi, 2016). The interruptions to metabolic activity in turn destroy senescent cells.

However, none of these apoptosis-targeting senolytic agents is universally effective to all types of senescent cells. Moreover, they inevitably have side-effects, for example, navitoclax also induces on-target thrombocytopenia, limiting their usage in clinic (Vogler et al., 2011). Furthermore, the lesson learnt from the acute clearance of tumor cells also implies some unexpected severe toxicity from senolysis via disrupting the physical functions of senescence in the body, like wound healing. Moreover, removing senescent cells from certain niches in the body such as stem cell niches, which are partially occupied by senescent cells, may induced stem cell proliferation, potentially leading to stem cell exhaustion or tumorigenesis. Thus, precautions are needed for the clinical use of senolysis therapy.

In summary, pro-senescence therapy is a promising new strategy for cancer treatment, but likely needs to be complemented with other types of therapies, such as immunotherapy and/or senolytic therapy for long term effects.

1.3 THE MYC ONCOPROTEIN

Apart from *RAS*, *MYC* is one of the best-documented oncogenes in cancer research. Due to its important role in cell proliferation, unconstrained expression of *MYC* is oncogenic.

Deregulated expression of MYC occurs in a large fraction of all human tumors, in particular at advanced stages and is often associated with poor prognosis (Dang, 2012). In fact, MYC is the most frequently amplified locus in human cancer (Beroukhim 2010). For example, MYC amplification occurs in 40% of basal-like breast cancer, 34% in ovarian cancer, 30% in lung adenocarcinoma and 29% in colon cancer (TCGA, The Cancer Genome Atlas). Therefore, it is important to uncover the precise role of MYC both in cell biology and tumorigenesis. The overview of MYC is summarized in Fig.2, which will be introduced in detail in the following sections.

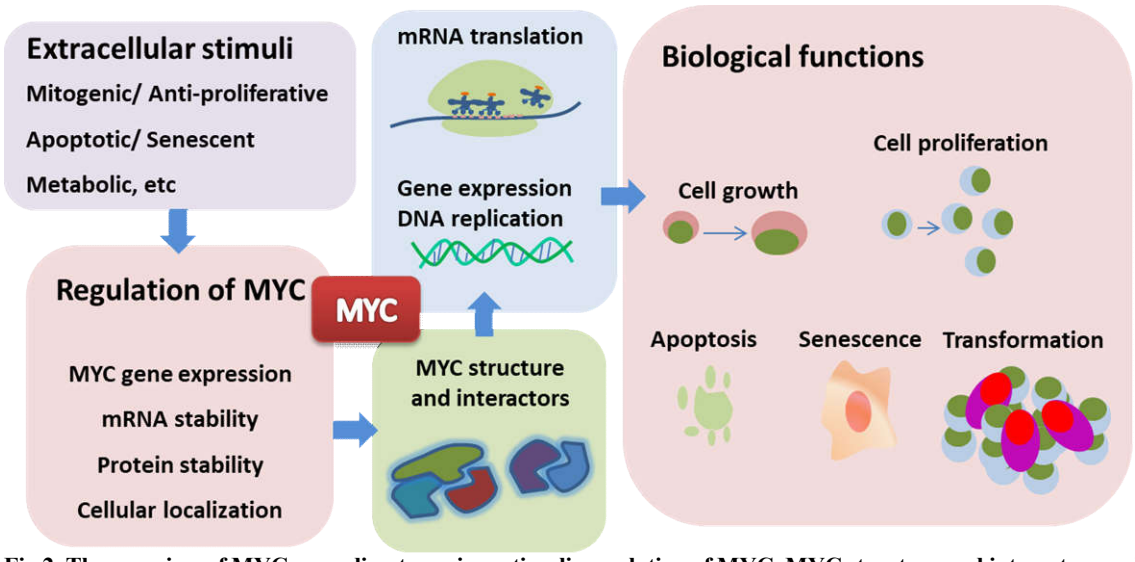

Fig.2. The overview of MYC regarding to various stimuli, regulation of MYC, MYC structure and interactors and MYC biological functions.

1.3.1 MYC structure and MYC interactors

The *MYC* (c-*MYC*) oncogene was originally discovered in the genome of avian myelocytomatosis virus MC29 (v-*myc*), in the form of a fusion gene between the viral structural GAG gene and the *MYC* sequence (Bister et al., 1977; Duesberg et al., 1979). It was then discovered that v-*myc* was derived from the chicken cellular *myc* proto-oncogene (c-*myc*) via retroviral transduction (Vennstrom et al., 1982). Later researchers found homologues of the *MYC* gene in all vertebrate genomes and eventually further down in evolution to all major metazoan linages (Young et al., 2011). Also two other *MYC* family members, MYCN and MYCL were discovered in the early 80s. Based on the *MYC*-related sequence, amplified *MYCN* was originally identified in human neuroblastomas and *MYCL* was found in human small cell lung carcinomas (SCLC) (Kohl et al., 1983; Nau et al., 1985; Schwab et al., 1983). MYC is quite ubiquitously expressed in proliferating mammalian cells,

the expression of MYCN and MYCL is more tissue-restricted during development and in adults. Therefore, the *MYC*-family genes (collectively referred to as *MYC* hereafter) have attracted many researchers' attention and is also the focus of this thesis.

MYC binds specifically to DNA, which is attributed to part of its highly conserved basic helix-loop-helix/leucine zipper (bHLHZip) domain in the carboxyl-terminus (Murre et al., 1989). The basic domain at the amino-terminal end of this region is responsible for specific binding to a DNA consensus sequence called E-box (CACGTG as similar sequences). The HLH and Zip motifs confer heterodimerization of MYC with another bHLHZip protein MAX, and this dimerization is necessary for DNA-binding and to serve as a transcription factor (Blackwell et al., 1990; Blackwood and Eisenman, 1991; Blackwood et al., 1992; Prendergast et al., 1991). The rest of the protein contains several evolutionary conserved so called MYC Boxes (MB) I-IV that also serves as interaction motifs with a wide range of proteins that play a role in MYC's diverse functions (Cole and Cowling, 2008; Cowling and Cole, 2006; Meyer and Penn, 2008). The MYC boxes will be mentioned later in different sections, but briefly; in the N-terminus, MBI harbors critical sites for MYC modifications (Thr 58 and Ser 62) that regulate MYC activation and degradation (Hydbring et al., 2010; Sears et al., 2000; Seo et al., 2008); MBII has been shown to interact with various of cofactors to regulate transcription, such as TRRAP and SKP2, and is required for transformation (Kim et al., 2003; McMahon et al., 1998; Park et al., 2002; von der Lehr et al., 2003; Wood et al., 2000); MBIII is related to transcriptional repression (Kurland and Tansey, 2008); and MBIV, which overlaps with the nuclear localization signal (NLS), is modified by acetylation and ubiquitylation and interacts with p300, FBXO28 and p27, and is implicated in MYC-driven transcription, transformation, apoptosis and G2 arrest (Adhikary et al., 2005; Bahram et al., 2016; Cepeda et al., 2013; Cowling et al., 2006).

1.3.2 Regulation of MYC

Unlike in cancer cells, MYC levels in normal cells are low even in proliferating cells. MYC is highly controlled both at the transcriptional and posttranslational levels (Levens, 2008). Regulation of MYC transcription is very complex, since it is activated by a number of mitogenic signal pathways, including receptor tyrosine kinases such as platelet-derived growth factor receptor (PDGFR) and epidermal growth factor receptor (EGFR), and downstream effectors RAS/RAF/MAPK, but also by WNT, Janus kinase/signal transducers and activators of transcription (JAK/STAT), sonic hedgehog (SHH), NOTCH and NF-κB pathways, which are often deregulated in cancer cells, leading to MYC overexpression (Clevers, 2004; Liu and Levens, 2006). MYC transcription is mainly initiated from two promoters, P1 and P2, where a variety of transcription factors can bind in response to different signals. Recently it is also reported that the MYC promoters are regulated from long distance by different super-enhancers, which are genomic regions containing multiple enhancers and collectively interacting with a variety of transcription factors (Cao et al., 2017). For instance, a CTCF-binding insulator region located 2 kb upstream of the MYC promoter,

has been shown to facilitate the interaction with a super-enhancer region around 2.8 Mb upstream of MYC (Schuijers et al., 2018). Moreover, MYC also represses its own expression through an autosuppression mechanism (Wierstra and Alves, 2008).

In terms of MYC posttranslational modifications, both Thr 58 and Ser 62 are important phosphorylation sites (Hydbring et al., 2017). Ser 62 has been reported to be phosphorylated by ERK, CDK1 and CDK2 in response to different signals, which can transiently stabilize and activate the MYC protein, resulting in induced transcription and proliferation (Benassi et al., 2006; Hydbring et al., 2010; Sears et al., 2000; Seo et al., 2008; Seth et al., 1991; Yeh et al., 2004). Moreover, Ser 62 phosphorylation is a prerequisite for Thr 58 phosphorylation. Thus, Ser 62 phosphorylation "primes" for Thr 58 mediated by GSK3β (Gregory et al., 2003; Lutterbach and Hann, 1994; Sears et al., 2000). GSK3β in complex with AXIN (Beurel et al., 2015), has been reported to recruit the PIN1 prolyl isomerase to restructure the phosphorylated Thr 58/Ser 62 region, thereby facilitating for protein phosphatase 2A (PP2A) to dephosphorylate Ser 62 (Yeh et al., 2004). MYC phosphorylated at Thr 58 is recognized by the F-box protein FBXW7, which is part of the SCF^{FBXW7} E3 ubiquitin ligase complex, leading to proteasomal degradation of MYC, growth inhibition and apoptosis (Arnold et al., 2009). MYC degradation through this pathway is regulated by AKT, which phosphorylates and inactivates GSK3β, thereby preventing GSK3β/FBXW7-mediated degradation of MYC (Cross et al., 1995; Manning and Toker, 2017). Besides SCF^{FBXW7}, other E3 ubiquitin ligases also ubiquitylate MYC, including SCF^{SKP2}, HUWE1/HECTH9 and SCF^{FBXO28}, which paradoxically activate MYC (Cepeda et al., 2013; Farrell and Sears, 2014; Kim et al., 2003; Thomas and Tansey, 2011; von der Lehr et al., 2003). On the other hand, mTOR has been implicated in regulating translation of MYC mRNA via both eIF4F and eIF4A-containing complexes (Bhat et al., 2015; Castell and Larsson, 2015; Wiegering et al., 2015; Wolfe et al., 2014). Similar to autosuppression at mRNA level, MYC also has negative feedback at the protein level, for example, by MYC-induced upregulation of Max's Next Tango (MNT), which competes with MYC for binding to MAX (Menssen and Hermeking, 2002).

1.3.3 MYC in transcription

Upon binding to E-boxes at target gene promoters, the MYC:MAX complex can activate transcription by recruiting different transcriptional cofactors with different functions. For instance, it has been reported that MYC regulates transcription in part via the modulation of histone modifications. Nucleosomes are the fundamental building blocks of chromatin, composed of 147 base pairs of DNA wrapped around a histone octamer composed of two copies of H2A, H2B, H3 and H4 (Grewal and Moazed, 2003). When nucleosomes are densely packed together, heterochromatin is formed, which limits the binding of transcription factor to DNA templates. In order to loosen up chromatin to make it more accessible or when going from open to condensed chromatin, histone modifications play an important role. In general, histone acetylation relaxes the chromatin to facilitate transcription factors binding and initiate transcription, which is mediated by histone acetyltransferases (HATs) and

reversed by histone deacetylases (HDACs). In addition, histone methylations on different lysines also affect transcription, such as H3K4me2/3 is related to active genes, and H3K9me3 and H3K27me3 are associated with gene repression, which are mediated through different histone lysine methyltransferase (HKMT), such as enhancer of zeste 2 (EZH2) and SUV39H1 (Bannister and Kouzarides, 2011). Importantly, MYC is engaged in this process. For example, TRRAP, which is associated with several large HAT complex, such as the GCN5-containing STAGA and the TIP60 complexes, is recruited to chromatin by MYC via MBII, and plays a role in chromatin relaxation and transcription activation (Liu et al., 2003; McMahon et al., 1998; McMahon et al., 2000). MYC can also repress gene expression through less understood mechanisms. For example, by binding the transcription factor MYC-interacting Zn finger protein-1 (MIZ-1), competes out the coactivator p300 and recruits HDAC3 and DNA methyl transferase 3a (DNMT3a) to the promoter regions of p15 and p21, leading to their repression (Brenner et al., 2005; Kurland and Tansey, 2008; Seoane et al., 2001; Staller et al., 2001; Wu et al., 2003).

Besides histone modifications, MYC is also shown to be involved in the alteration of nucleosome structure. The structure and positioning of nucleosomes can be altered by different ATP-dependent chromatin remodeling complexes, including five families in eukaryotes, i.e. SWI/SNF, nucleosome remodeling deacetylase (NuRD), ISWI, INO80 and SWR1. It has been reported that MYC-mediated transactivation requires the recruitment of a component of the SWI/SNF complex SNF5/INI1 at bHLH region, indicating SWI/SNF complex also assists MYC-mediated chromatin remodeling (Cheng et al., 1999).

By binding to different cofactors, MYC is involved in different steps of the transcription process. MYC has been shown to promote transcription factor IIH (TFIIH) activity to phosphorylate on C-terminal domain (CTD) Ser 5 (S5) of RNA Polymerase II (RNAP II), leading to transcription initiation (Cowling and Cole, 2007). Recently, a role of MYC in transcription elongation has been suggested. After transcription initiation, RNAP II is pausing at approximately 30-50 bp downstream of transcription start site (TSS) of many genes. It was reported that binding of MYC:MAX to target gene promoters results in promoter clearance and elongation through the recruitment of P-TEFb (Bouchard et al., 2004; Eberhardy and Farnham, 2001, 2002; Rahl et al., 2010).

MYC also recruits several E3 ubiquitin ligases to chromatin, including SCF^{SKP2}, HUWE1/HECTH9 and SCF^{FBXO28}, which ubiquitylate MYC in a proteolytic or non-proteolytic manner, but also play a role in promoting MYC-driven transcription (Adhikary et al., 2005; Cepeda et al., 2013; Kim et al., 2003; von der Lehr et al., 2003). Recently, it was reported that this not only increases histone acetylation, but also transfers RNAP II-associated factor complex (PAF1C) to RNAP II to stimulate elongation (Jaenicke et al., 2016).

Since the identification of MAX and the E-box binding activity of the MYC:MAX heterodimer, the consensus model has been that MYC is working gene-specifically, i.e. MYC:MAX complex is bound to specific E-box-containing genes to regulate transcription. In contrast, more recent studies suggested that MYC works as a global amplifier of transcription

(Lin et al., 2012; Nie et al., 2012). However, other research groups challenged this theory. Bruno Amati's group reported that MYC primarily acts through differential regulation of its direct target genes, and that global RNA amplification is an indirect effect of activation of these specific genes, which initiates a cascade of downstream events (Sabo et al., 2014). Martin Eilers´ group also confirmed that transcriptional amplification occurred as a result of MYC:MAX binding to a specific set of genes, that either leads to activation or repression of gene transcription, in part dependent on the interaction with MIZ-1 (Walz et al., 2014). Though these different models are still under debate, there is a general agreement that MYC plays an essential role of in regulating genes affecting many cellular processes, including cellular proliferation, growth, apoptosis, DNA damage and senescence.

1.3.4 MYC in cell cycle and proliferation

MYC expression is highly related to cell proliferation. Early studies in quiescent lymphocytes and rodent fibroblasts showed that *MYC* mRNA was promptly activated within 2 hours after mitogenic stimulation. This is typical for immediate early response genes such as FOS and JUN (Dean et al., 1986; Kelly et al., 1983; Reed et al., 1986). Further, quiescent rodent fibroblasts can re-enter cell cycle by ectopic expression of MYC even in the absence of serum factors, for instance by using the regulatable 4-hydroxytamoxifine-induced MycER system, showing that MYC is sufficient to induce cell cycle entry and progression (Eilers et al., 1991). Moreover, MYC is important for maintenance of the cell cycle, since depletion of MYC leads to cell cycle arrest in a variety of cell lines (Wang et al., 2008). MYC contributes to cell cycle progression through its transcriptional function; firstly, many factors encoded by MYC-target genes, e.g. cyclin D2 (CCND2), cyclin E1 (CCNE1), E2F1, and others promote this process. Secondly, MYC also inhibits the expression of CDK inhibitors p15, p21 and p27, e.g. through the assistance of MIZ-1 (Claassen and Hann, 2000; Seoane et al., 2002; Staller et al., 2001; Wu et al., 2003).

MYC also plays an essential role in S phase via by stimulating DNA replication. It has been reported that MYC can physically interact with the prereplicative complex (pre-RC) proteins CDC6 and minichromosome maintenance (MCM) proteins (Dominguez-Sola et al., 2007). Besides regulation of CDKs, which are involved in origin firing, MYC also favors opening of chromatin, which permits the loading of replication- related complexes (Dominguez-Sola and Gautier, 2014). Moreover, MYC can directly promote the recruitment of CDC45 to chromatin, leading to initiation of DNA replication (Dominguez-Sola et al., 2007; Sankar et al., 2009; Srinivasan et al., 2013).

1.3.5 MYC in growth and metabolism

Activation of cellular growth and cell cycle progression require dramatic changes in cellular metabolism, in which MYC also plays a major role. MYC is associated with different aspects of metabolism, including nutrient sensing and uptake, ribosome biogenesis, protein synthesis,

nucleotide synthesis and energetic metabolism (van Riggelen et al., 2010). First, MYC plays a critical role in glycolysis and glutaminolysis. MYC enhances uptake of glucose and glutamine via induction of glucose and glutaminase transporters. Moreover, MYC also activates other related factors. For example, MYC-induced monocarboxylate transporter 1 (MCT1) is responsible to export lactate after conversion from glucose. Besides, MYC also promotes RNA splicing of pyruvate kinase M2 (PKM2) over PKM1, favoring glycolytic intermediates to be used in other biosynthesis pathways (Dang, 2013; Stine et al., 2015). In addition, MYC is also important for ribosome biogenesis and protein synthesis. In terms of ribosome biogenesis, first, among a wide range of MYC-induced RNAP II-transcribed genes, there are many mRNA encoding ribosomal protein (Fernandez et al., 2003; Orian et al., 2003). In addition, MYC also promotes transfer RNA (tRNA) expression via interaction with RNAP III transcription apparatus (Gomez-Roman et al., 2003). Besides, MYC also facilitates RNAP I-mediated ribosomal RNA (rRNA) transcription, thereby coordinating the activity of all three RNA polymerases (Arabi et al., 2005; Grandori et al., 2005). mRNA translation is also stimulated by MYC through increased expression of CAP-dependent translation initiation factors, e.g. eIF4E, eIF2α, eIF4AI and eIF4GI (Arabi et al., 2005; Schlosser et al., 2003; Schmidt, 2004). However, eIF4E stability is also prolonged by eIF4E binding protein 1 (4EBP1), which is regulated by mTOR, another major regulator of protein synthesis besides MYC (Sonenberg and Hinnebusch, 2009). Therefore, eIF4E connects MYC and mTOR signaling. Moreover, MYC depletion suppresses deoxyribonucleoside triphosphate (dNTP) metabolism, via the reduction of corresponding rate-limiting enzymes, such as thymidylate synthase (TS), inosine monophosphate dehydrogenase 2 (IMPDH2) and phosphoribosyl pyrophosphate synthetase 2 (PRPS2) (Mannava et al., 2008). MYC also favors catabolism of excessive glutamine in order to sustain protein and nucleotide biosynthesis (Dang, 2010). Further, MYC also promotes mitochondrial biogenesis. It has been reported to regulate at least 18 mitochondrial genes and 400 nuclear genes encoding mitochondrial proteins, indicating an intricate link between MYC and mitochondrial biogenesis and metabolism (Coller et al., 2000; Kim et al., 2008; Schuhmacher et al., 2001; Seitz et al., 2011). Since the cell cycle, cell growth and metabolism need to be coordinated, it is obvious that MYC does not only activate expression of proliferative genes and genes involved in protein, nucleotide and lipid synthesis, but also accelerates the mitochondrial activity, such as increased oxygen consumption, mitochondrial membrane potential as well as mitochondrial mass, in order to support cell proliferation (Morrish et al., 2008; Schuhmacher et al., 1999).

1.3.6 MYC in DNA damage response

MYC overexpression can cause DNA damage via at least two types of routes. One is through the production of ROS, which is either directly induced as a consequence of MYC activity or indirectly by p53 activation in response to abnormal MYC expression (Polyak et al., 1997; Vafa et al., 2002). The other route is via replication stress. This is due to the deregulated S-phase entry upon uncontrolled MYC expression, leading to replication fork stalling or collapse (Gorgoulis et al., 2005; Srinivasan et al., 2013). As a result of DNA damage, DDR is

triggered, majorly via ATM-CHK2 or ATR-CHK1 pathway (Maclean et al., 2007; Pusapati et al., 2006). The purpose of DDR is to repair damaged DNA to prevent mutations, however, if the damage is severe, this cell will undergo apoptosis or senescence in order to keep organism in homeostasis. If the DNA repair, apoptosis or senescence systems are deficient, it will result in genomic and chromosomal instability, eventually leading to tumorigenesis.

1.3.7 MYC in apoptosis

While promoting cell proliferation, MYC is also a potent inducer of apoptosis (Evan et al., 1992). MYC is directly involved in both the intrinsic and extrinsic apoptosis pathways. For example, in the intrinsic pathway, MYC overexpression lead to suppression of anti-apoptotic genes *BCL*-2 and *BCL*-X_L but induction of the pro-apoptotic genes *BAX* and *BIM*, thereby contributing to release of cytochrome C, caspase activation and apoptosis (Eischen et al., 2001; Youle and Strasser, 2008). In addition, in the extrinsic pathway, MYC sensitizes cells to TNF-α-induced apoptosis, and promotes the association between BID and mitochondria to generate truncated BID (tBID), sequentially resulting in activation of pro-apoptotic BAX and BAK (Nilsson and Cleveland, 2003). Moreover, the p53 pathway also participates in MYC-induced apoptosis. Firstly, MYC induces *ARF* expression, and the latter sequesters MDM2, which is involved in degradation of p53, thereby leading to stabilization of p53. Meanwhile, MYC also suppresses *p21*. In turn, the lack of p21 promotes the apoptotic function of p53 over its cytostatic function (Henriksson et al., 2001; Li and Hann, 2009). Therefore, in order to overcome MYC-induced apoptosis, it is common that MYC overexpression in aggressive tumors is accompanied with BCL-2 upregulation (Martin-Subero et al., 2005; Mukhopadhyay et al., 2005; Seoane et al., 2002). In addition, it is very frequent that MYC-driven tumors carry mutant p53 (Henriksson et al., 2001; Nilsson and Cleveland, 2003). It has also been reported that more modest MYC levels in pre-malignant lesions promotes proliferation and transformation, while too high MYC levels will result in apoptosis, thereby aborting tumorigenesis (Murphy et al., 2008). However, disruption in apoptotic pathways, e.g. p53 mutation or BCL2/BCL-X_L overexpression, later in tumor development will allow higher MYC expression that will fuel high proliferation and aggressive tumor development.

1.3.8 MYC in senescence regulation

As mentioned before, activated RAS is known as a potent inducer of senescence trigger when overexpressing. However, MYC has been reported to counteract RAS-induced senescence in primary rodent cells (Hydbring et al., 2010; Larsson and Henriksson, 2010), which was shown to be dependent on CDK2-mediated phosphorylation of MYC at Ser 62 (Hydbring et al., 2010). Moreover, RAS also overcome MYC-induced apoptosis via activation of the PI3K/AKT pro-survival pathway. Therefore, cooperativity in rodent cells between MYC and RAS overrides two barriers of tumorigenesis-senescence and apoptosis, which contributes to transformation. Moreover, MYC expression can also overcome $BRAF^{V600E}$-induced senescence *in vivo*, accelerating mouse lung tumor development (Tabor et al., 2014) as well

as BRAF- or NRAS-induced senescence in human melanoma cells (Zhuang et al., 2008). Further, it has been observed that MYC inactivation/depletion results in cell proliferation arrest, apoptosis and/or senescence in different experimental cancer models both *in vitro* and *in vivo*. This has led to the concept of MYC oncogene 'addiction' in certain tumors, where cancer cells become dependent on an overdose of MYC in cancer-related cellular processes compared with normal cells (Boxer et al., 2004; Felsher, 2010). The underlying mechanism of MYC downregulation-mediated senescence remains unclear (Wu et al., 2007; Zhuang et al., 2008). For example, the senescence by MYC-inactivation in osteosarcoma is independent on RAS-MAPK pathways and DNA damage response, which often play important roles in senescence regulation (Wu et al., 2007). Additionally, melanoma cells can also undergo senescence upon MYC depletion independent of two essential players for senescence-p53 or p16 (Zhuang et al., 2008). However, suppression of senescence by MYC seems to be cell context-dependent. In Werner syndrome fibroblasts, which lack of DNA repair function due to a mutation in *WRN* gene, MYC overexpression induces rather than suppresses senescence due to increased accumulation of DNA damage (Grandori et al., 2003). Another example is CDK2 knockout mouse embryonic fibroblasts (MEFs), where MYC induces senescence independent of DNA damage (Campaner et al., 2010). This may be seemingly contradictory with Hydbring et al., where CDK2 stimulated MYC's suppression of senescence (Hydbring et al., 2010). Possibly, CDK2 acts as a switch, turning MYC from an inducer (when CDK2 is inactive) to a suppressor (when CDK2 is active) of senescence.

1.3.9 MYC in immune response

In addition to cell-intrinsic effect, such as promoting cell proliferation and cell growth, MYC overexpression has also been associated with tumor immune evasion. There are several studies revealing that MYC and MYCN regulate the gene expression of immune checkpoints-cluster of differentiation 47 (*CD47*, assisting cells to avoid phagocytosis (Majeti et al., 2009)) and *PD-L1* (enhancing immune tolerance via binding to PD-1 (Tumeh et al., 2014)), leading to escaping immunosurveillance and promoting tumorigenesis in different tumor models, such as lymphoma, liver cancer, lung cancer and neuroblastoma (Atsaves et al., 2017; Casey et al., 2016; Kim et al., 2017; Melaiu et al., 2017). This effect can be reversed by interfering with MYC level, such as short hairpin-mediated MYC knockdown or BET inhibitor JQ1-induced MYC repression, followed with restoration of immune response and tumor regression. MYC has been shown to regulate the expression of *CD47* and *PD-L1* via directly binding to their promoters. These studies confirmed that MYC oncogenic function is not only through promoting tumor cell proliferation but also through preventing tumor cell from immune attack, which uncovered the new facet of MYC in tumorigenesis. However, in order to obtain a precise view of MYC function in immune response, more studies need to be carried out. First, it is unclear whether this immune-related role of MYC is an oncogenic requirement or a physiological process. The occupancy of MYC in the promoters of *CD47* and *PD-L1* occurs at a high MYC level, which would be due to 'promoter invasion', as a sign for oncogenic event. However, this may also happen in normal development of rapid cell

expansion, which upregulates NKG2D and is a risk to be attacked by immune system, therefore, MYC-mediated immune suppression is also required for rapid normal cell proliferation. Another thing is to clarify whether this is the only mechanism for MYC to suppress immune system or there are other routes. One possibility is through cytokines regulated by MYC, such as thrombospondin-1, involved in angiogenesis and senescence (Rakhra et al., 2010), and type 1 IFN (Schlee et al., 2007), associated with both innate and adaptive immunity. It has also been shown that in the $KRAS^{G12D}$-driven adenomas, MYC overexpression not only enhanced *PD-L1* expression, but also promoted the secretion of CCL9 and IL-23 in stromal cells to suppress immune system, switching to proliferative and invasive adenocarcinomas (Kortlever et al., 2017). In addition, these immune suppressive properties also exist in other oncogenes, such as BRAF and β-catenin (Spranger et al., 2015; Sweis et al., 2016; Wilmott et al., 2012), which may converge to MYC- or other more parallel pathways-mediated immune suppression.

1.3.10 MYC inhibitors

Due to the predominant role of MYC in tumorigenesis, MYC seems to be an ideal therapeutic candidate. Therefore, researchers have tried to find ways to interfere with MYC either directly or indirectly. Because MYC is an intrinsic disordered protein and lack enzymatic activity, proteins like MYC are difficult to target. However, MYC function is dependent on binding to its obligated partner MAX. Thus, a possible strategy for targeting MYC is to target the MYC:MAX interaction. Quite some effort has been made from different labs, and several small molecules have been reported to inhibit MYC:MAX interaction, such as IIA4B20, 10058-F4 and Mycro1 (Berg et al., 2002; Kiessling et al., 2006; Yin et al., 2003). But none of these compounds can be used clinically due to limitations, such as low potency in cells or *in vivo* or poor specificity (Fletcher and Prochownik, 2015; McKeown and Bradner, 2014; Prochownik and Vogt, 2010; Whitfield et al., 2017). However, our lab has identified and characterized two MYC:MAX inhibitors, MYCMI-6 and MYCMI-7, which displayed good specificity and efficacy both *in vitro* and *in vivo* and are presented in paper III (Castell et al., 2018) and paper IV. There are also inhibitors reported to targeting MYC transcription, for instance BET bromodomain inhibitors such as JQ1 (Delmore et al., 2011) and I-BET (Chaidos et al., 2014; Dawson et al., 2011). BET proteins recognize acetylated histones within chromatin, and play a role in the recruitment of transcriptional regulators to activate gene transcription. In a certain context, such as murine leukaemia and multiple myeloma cells, BET proteins was reported to regulate the *MYC* gene directly, thus, upon BET inhibitors treatment, MYC expression was repressed (Dawson et al., 2011; Delmore et al., 2011; Filippakopoulos et al., 2010). However, in B-cell lymphomas, JQ1 treatment caused tumor suppression independent on the MYC level (Bhadury et al., 2014; Donato et al., 2017). Alternatively, exploiting MYC for synthetic lethality approaches could also contribute to anti-cancer therapy. Synthetic lethality is where deficiencies in two or more genes together causes cell death, while inactivation of either one alone does not. There are several reports that deregulation of MYC can be exploited to cause synthetic lethality. For example, since

overexpression of MYC causes replicative stress, targeting the DNA damage pathway, including proteins such as ATR and CHK1, results in accumulation of additional genomic instability and cause cell death (Cottini et al., 2015; Dominguez-Sola and Gautier, 2014). Moreover, since MYC reprograms cellular metabolism, thereby sensitizing cell to disturbances in metabolic alterations, inhibition of glutaminase has been reported to exert synthetic lethality with MYC, leading to cell death (Gao et al., 2009; Qing et al., 2012; Yuneva et al., 2007). Moreover, as mentioned above, inhibition of CDK2 in the context of MYC overexpression causes cellular senescence (Campaner et al., 2010; Hydbring et al., 2010) or apoptosis (Molenaar et al., 2009). Undoubtedly, with a deeper understanding of the role of MYC in cell and tumor biology, different strategies will be developed in order to target MYC, eventually combating MYC-driven cancer.

2 AIMS

The overall aim of this thesis was to elucidate the potential of targeting MYC and other proteins connected to the MYC network for pro-senescence therapy as an alternative anti-cancer treatment. Part of that task has been to elucidate the role of MYC in senescence regulation in normal human and malignant cells. Another part was to identify and characterize small molecules specifically targeting MYC in a number of cancer cell lines.

The specific aims of each paper are as follows:

Paper I: To explore the role of MYC in regulation of $HRAS^{V12}$-induced senescence in human normal fibroblasts.

Paper II: To evaluate whether pro-senescence therapy could be an alternative strategy in melanoma treatment utilizing established and experimental targeting drugs.

Paper III and IV: To characterize the small MYC:MAX inhibitory molecules MYCMI-6 and MYCMI-7 with respect to efficacy and selectivity, and their potential in inhibiting MYC-driven tumor cell growth.

Paper V: To investigate the potential of experimental, pharmacological MYC inhibitors to induce senescence in melanoma cells, alone or in combination with other drugs.

3 RESULTS AND DISCUSSION

3.1 PAPER I: MYC AND RAS ARE UNABLE TO COOPERATE IN OVERCOMING CELLULAR SENESCENCE AND APOPTOSIS IN NORMAL HUMAN FIBROBLASTS

MYC and RAS are two of the best-documented oncogenes in tumorigenesis. However, they also trigger intrinsic tumor suppressor mechanisms, i.e. apoptosis and senescence, respectively, in order to dampen their tumorigenic function. From studies of rodent cells, MYC and RAS can cooperate with each other to overcome each other's limitations. Coexpression of MYC and RAS is sufficient for transformation of primary rodent cells, but is, however, not sufficient for transformation of human primary cells, where additional oncogenic events are required. However, since overcoming apoptosis and senescence is necessary but not sufficient for transformation, it remains unclear whether in human primary cells, MYC and RAS still collaborate to overcome these fail-self mechanisms as in rodent cells.

In **Paper I**, to explore this possibility, we utilized human primary BJ fibroblasts cells with tetracycline-induced $HRAS^{V12}$ and 4-hydroxytamoxifen (OHT)-regulated MycER system. In this system, doxycycline (DOX) binds to reverse tetracycline-controlled transactivator (rtTA), leading to transactivation of the $HRAS^{V12}$ gene, which is under the control of a promoter containing tet response element (TRE) (Evangelou et al., 2013; Maya-Mendoza et al., 2015). In MycER, the *MYC* gene is fused in frame to the ligand-binding domain of the estrogen receptor (ER). In the absence of OHT, chaperone proteins keep MycER in an inactive state. These proteins will be released upon addition of OHT, leading to activation of the MycER protein (Littlewood et al., 1995).

HRAS-induced senescence was confirmed in BJ cells upon DOX treatment, with cell proliferation and cell cycle arrest, enlarged cell size and increased SA-β-GAL activity, as well as increased expression other senescence-related biomarkers, such as H3K9me3, p21 and p16, and reduced phosphorylation of pRB. Upon MYC activation apoptosis was strongly induced, resulting in decreased cell number, increase of the percentage of dead cells, induction of DNA damage, p53 and increased PARP cleavage. Upon the dual induction of MYC and RAS, H3K9me3 and SA-β-GAL activity decreased somewhat, the expression of p16 increased further, compared to RAS induction alone. Therefore, MYC was unable to fully override RAS-induced senescence. In addition, MYC activation also reduced the level of RAS, as well as the downstream effectors phospho-ERK (P-ERK) and phospho-AKT (P-AKT), suggesting RAS pathway was tuned down by MYC. On the other hand, MYC-triggered apoptosis remained at the same level after dual induction of MYC and RAS. This was not dampened by tuning down the OHT concentration or by scheduling the start of MYC activation to different time points after of RAS induction. Thus, RAS failed to overcome MYC-induced cell death in BJ cells.

Given the role of p53 in oncogene-induced apoptosis and senescence, and the upregulation of p53 upon MYC induction in our results, we assumed that p53 loss would be a possible way to rescue these cells. After establishment of a p53 knockdown cell line, we observed RAS-induced senescence was overcome by p53 depletion, but MYC-triggered apoptosis was still not affected to any major extent, as shown by increased cleavage of PARP and cell death.

It is known from previous work that MYC and RAS fail to transform human primary cells. In summary, our work now shows that MYC and RAS are unable to cooperate in overcoming even the first barriers of transformation, i.e. apoptosis and senescence. The main obstacle is apparently MYC-triggered cell death. A possible explanation is that the MYC-induced DDR signal is too strong and cannot be tolerated by cells, while the magnitude of pro-survival signaling, such as the RAS-induced PI3K/AKT pathway is still not sufficient or strong enough to abrogate MYC-induced cell death. One explanation for this is our observation that MYC activation dampened RAS-induced PI3K/AKT pathway. It has also been reported that activation of ERK can exert negative feedback signals to inhibit the RAS/MAPK pathway, which can be accelerated upon MYC induction (Shin et al., 2009). Hence, another possibility would be this negative feedback is stronger in human cells than in rodent cells, leading to the downregulation of RAS-signaling. Further, since ERK can phosphorylate MYC at Ser 62, which is important for survival upon DNA damage and for suppression of senescence by MYC (Benassi et al., 2006; Hydbring et al., 2010; Hydbring et al., 2017), down-modulation of RAS/MEK/ERK-signaling may restrain MYC function, leading to a vicious cycle. Additionally, the failure of p53 depletion to rescue MYC-induced cell death implies other factors, such as BCL2 and BCL-X_L are required to override this aspect of MYC signaling in the context of human primary cells. Therefore, in human primary BJ fibroblasts, MYC and RAS failed to cooperate to reciprocally overcome cell death and senescence, and this was not affected by p53 knockdown.

3.2 PAPER II: PRO-SENESCENCE THERAPY - A NEW STRATEGY TO OVERCOME DRUG RESISTANCE AND ENHANCE IMMUNORECOGNITION OF MALIGNANT MELANOMA CELLS

The concept of therapy-induced senescence is built on observations that cancer cells often have an intact senescence induction system and retain the potential to senesce. Hence, based on to the tumor-suppressive and immunogenic function of senescence, we have utilized different established and experimental targeting drugs to address the question whether senescence can be established in human melanoma cells with different genetic backgrounds, and if so, whether there are differences in senescence phenotypes, SASP profiles and immune receptor expression of relevance for immunosurveillance between different drugs and/or genotypes, and finally, if pro-senescence therapy is a promising strategy for treatment of melanoma.

In **Paper II**, to explore the potential of pro-senescence therapy in melanoma, we performed a senescence screen using a panel of eleven melanoma cell line with different common driver

mutations such as $BRAF^{V600E}$, $NRAS^{Q61R}$, mutations enhancing the activity of PI3K or CDK4, or loss of PTEN or p53 activity. Ten selected targeted clinical and preclinical drugs of relevance for these mutations, such as vemurafenib ($BRAF^{V600E}$ inhibitor), trametinib (MEK1/2 inhibitor), BKM-120 (PI3K inhibitor), RITA (p53:MDM2 inhibitor), APR-246 (re-activator of mutated p53) and palbociclib (CDK4/6 inhibitor), together with DNA alkylating agent temozolomide, which is a conventional drug used in melanoma therapy. Cells were seeded in 384-well plates, followed by three-day single drug treatment and immunofluorescent staining of several senescence-related markers were selected for the screen, including cell number, 5-ethynyl-2'-deoxyuridine (EdU) incorporation, cell area, nuclear area, intensity of H3K9me3 and p53 staining, as well as the immune marker HLA class I.

Vemurafenib and trametinib displayed a good senescence response in two of four cell lines with $BRAF^{V600E}$ mutation, A375 and ESTDAB-049, while SKMEL28 and in particular ESTDAB-037 showed a poor response, indicating an intrinsic resistance to vemurafenib and trametinib in the latter. Vemurafenib were not effective for non-BRAF mutated cells as expected. Though trametinib suppressed cell growth in cells with NRAF mutation, the senescence response was not as good as the responsive BRAF-mutated cell lines. Temozolomide at conditions used was not sufficient to induce senescence in all cells. Interestingly, palbociclib, crizotinib and BKM120 arrested cell proliferation and induced senescence to different extent in all cells independent of BRAF/NRAS mutation. In terms of p53 activators, nutlin-3A and RITA induced senescence in most cell lines with wild type p53 as expected, while APR-246 affected most cell lines wild type p53 and some with p53 mutation. HLA class I expression was more evident upon palbociclib treatment in all cell lines except ESTDAB37 and KADA, and was enhanced upon crizotinib or nutlin-3A treatment in many cells. Thus, we found three drugs (palbociclib, crizotinib and BKM120) induced growth arrest and senescence in most of cell lines irrespective of their BRAF/NRAS status.

Next we selected vemurafenib, trametinib, palbociclib and BKM120, for further validation using two BRAF mutant cell lines, A375 and ESTDAB-037, the NRAS mutant cell line SKMEL2 and two non-BRAF/NRAS mutated lines, ESTDAB-140 and KADA. The following markers were measured: cell number, cell cycle distribution, cell size, nuclear size, percentage of dead cells, SA-β-GAL activity, expression of H3K9me3, p53, phosphorylated pRb, cyclin B, cleaved caspase 3 and SASP factors. It was confirmed that vemurafenib induced senescence in A375 but not in the ESTDAB37 BRAF-mutated line, and not in other cell lines. Trametinib, like vemurafenib induced senescence in A375, but also induced some markers of senescence in ESTDAB37. In the NRAS-mutated line SKMEL2, trametinib triggered many markers of senescence but almost no change in EdU incorporation or cell cycle distribution and also triggered cell death, suggesting a quite complex phenotype. Further, senescence induction was poor in the non-BRAF/NRAS-mutated cell lines after trametinib treatment. The PI3K inhibitor BKM120 affected some but not all markers of senescence in all cell lines, although the markers induced sometimes difference between cell

lines. Finally, palbociclib induced all senescence markers in all cell lines with few exceptions, irrespective of BRAF/NRAS status. Moreover, unlike most of the other drugs, palbociclib did not induce cell death, which makes palbociclib more of a general senescence inducer.

We also examined the expression of SASP factors in response to the treatments, including AREG, CSF2, CXCL1, IL-1α, IL-1β, IL-6, IL-6R, IL-8 and MMP3. Among these factors, vemurafenib and trametinib induced only IL-6 in A375 but not in ESTDAB37 or other cell lines, except SKMEL2 where trametinib increased IL-6 and some other cytokines. BKM120 and palbociclib had a much broader effect on SASP factor expression in most cell lines, but preferentially induced IL-1α/β, sometimes with CXCL1 and IL-8, while induction of AREG seemed to be more specific for BKM120. We also confirmed HLA class I expression, where vemurafenib only induced HLA class I in A375, while trametinib induced HLA class I in A375, ESTDAB37 and SKMEL2. BKM120 as well as palbociclib increased HLA1 in all cells, implying a potential for increased immunogenicity.

We next wondered whether any combination treatment could overcome resistance to vemurafenib-induced senescence in $BRAF^{V600E}$-mutant melanoma cell lines as well as resistance to trametinib. Therefore, we performed a second combination treatment senescence screen in 96-well plate format for three-day treatment, including vemurafenib or trametinib combined with each of all the other drugs at a serial dilution in five cell lines (A375, A375-VR4 (acquired resistance to vemurafenib), ESTDAB-037, ESTDAB-140 and KADA). Subsequently, cell number, cell size and EdU incorporation was measured by immunofluorescent staining and imaged under a fluorescence microscope. The results indicated that palbociclib, crizotinib and BKM120 can synergize with vemurafenib to trigger senescence in both vemurafenib-sensitive and –resistant cell lines.

Finally, since palbociclib, crizotinib and BKM120 alone induced senescence in most melanoma cell lines irrespective of BRAF/NRAS status, we were interested in whether combination of any of these drugs would synergize in senescence induction. We found that in particular the combination of palbociclib and crizotinib further enhanced senescence , which was confirmed by increased SA-β-GAL activity, reduced phosphorylated pRb and arrested proliferation in all the five cell lines. Further, the expression of certain SASP factors, preferentially on IL-1α/β, was increased with this drug combination irrespective of BRAF/NRAS mutation status. In addition, we also evaluated immune-related receptors for natural killer (NK) and T-cell recognition on A375, A375-VR4 and KADA cells. Upon palbociclib treatment, both HLA class I and class II, responsible for antigen-presenting to $CD8^+$ and $CD4^+$ T cells, respectively, were enhanced. The combination treatment further increased these two markers in KADA cells, but not in A375 and A375-VR4 cells. Moreover, PD-L1, inhibiting T cell function, was increased upon palbociclib treatment and further enhanced or stay the same level upon dual treatment. This suggested the combination treatment upregulated receptors for both T cell recognition and T cell suppression. In addition, ULBP2/5/6, ligands of NKG2D NK cell receptors, were also enhanced upon palbociclib

treatment and increased more with the addition of crizotinib in A375 and KADA, indicating combination also enhanced NK cell recognition.

In summary, we found that palbociclib, BKM120 and crizotinib had the potential to trigger senescence in many cell lines irrespective of BRAF/NRAS mutations in our screen. In addition, these three drugs also were capable to overcome resistance to vemurafenib and trametinib. Moreover, upon the combination treatment of palbociclib and crizotinib further enhanced senescence in most of melanoma cell, linked to increased SASP factor expression and regulation of immune receptors of relevance for killing by T cells and NK cells.

Pro-senescence therapy as an alternative strategy for anti-cancer therapy is under debate. In particular, the role of SASP factors needs to be clarified further. There are a number of publications arguing for the benefit of SASP factors. For example, different SASP factors, such as CCL2, TNFα and ICAM-1, can recruit NK cells and M1-like macrophages to clear senescent tumor cells (Iannello et al., 2013; Lujambio et al., 2013; Ruscetti et al., 2018; Xue et al., 2007). Moreover, dual inhibition of AURKA and MDM2 was reported to induce CCL5 expression, leading to tumor clearance by the immune system in lymphoma model (Vilgelm and Richmond, 2015). However, the SASP is heterogeneous and for the most part pro-inflammatory, which can cause chronic inflammation and promoter cancer development. It would therefore be interesting to validate our combination of drugs both *in vitro* and *in vivo*. Using immune cell killing assays one could investigate whether modulation of the protocol, for instance by including immunotherapy or a third targeted therapy, would improve the efficacy of the pro-senescence therapy. One should also investigate the immune response in melanoma mouse models *in vivo* in the future.

3.3 PAPER III AND PAPER IV: CHARACTERIZATION OF SELECTIVE INHIBITORS FOR MYC:MAX INTERACTION MYCMI-6 AND MYCMI7 AND EVALUATION THE POTENTIAL IN INHIBITING MYC-DRIVEN TUMOR CELL GROWTH

Given that MYC plays a crucial role in tumorigenesis with its partner MAX, we focused on developing inhibitors targeting the MYC:MAX interaction. Thus, we first performed a cell-based screen for inhibitors of MYC:MAX interaction. The benefit with cell-based screens is that one can identify cell-permeable compounds with inhibitory activity in a cellular context already in the screening step, which is not possible using *in vitro* approaches. The screen was based on bimolecular fluorescence complementation (BiFC), in which full-length MYC and MAX were fused with two fragments of yellow fluorescent protein (YFP) separately. After transfection, MYC:MAX interaction will bring YFP fragments in close proximity in cells (Grinberg et al., 2004; Hu et al., 2002; Kerppola, 2006; von der Lehr et al., 2003). Through the screen, we found six compounds (MYCMI-2, MYCMI-6, MYCMI-7, MYCMI-9, MYCMI-11 and MYCMI-14) potentially targeting MYC:MAX interaction without affecting the BiFC interaction between the bZip transcription factors FOS and JUN. Two of them,

MYCMI-6 and MYCMI-7 were further characterized in **Paper III** and **Paper IV**, respectively.

We also set up another protein-fragment complementation assay (PCA) assay for validation of these six compounds, in which full-length MYC and MAX were separately fused with two parts of the Gaussia luciferase (GLuc). Unlike BiFC assay, the interaction between these two fragments of luciferase are complementary but reversible, and emit a luminescence signal after catalyzing the cell permeable substrate coelenterate luciferin (Remy and Michnick, 2006). All six compounds showed better inhibitory effects on both MYC:MAX and MYCN:MAX interactions at a concentration of 25 μM, compared to the previously reported MYC:MAX inhibitor 10058-F4 at 64 μM (Huang et al., 2006). None of the compounds affected homodimerization of the bZip transcription factor GCN4, thus confirming the selectivity of the compounds for MYC:MAX. Two of the molecules, MYCMI-6 and MYCMI-7, were the most efficient MYC:MAX inhibitors, and are the focus of **Paper III** and **Paper IV**, respectively. Since MYCMI-7, but not MYCMI-6, decreased the endogenous MYC level, the two molecules seem to work through different mechanisms. In **Paper III**, we focused on molecules that inhibit the MYC:MAX interaction without affecting MYC expression, and in **Paper IV** on MYCMI-7 that does both.

In Paper III, we further validated MYCMI-6, MYCMI-11 and MYCMI-14. The three compounds targeted endogenous MYC:MAX interaction as shown by in situ proximity ligation assay (isPLA). In isPLA, after a pair of primary antibodies bind to the two interacting proteins of interest, secondary antibodies conjugated with DNA oligos are bound to primary antibodies, bringing the oligonucleotides in close proximity, provided that the two proteins interact in the cell. This allows hybridization between oligos and formation of a circular DNA template containing nicks, which are ligated together. After rolling circle amplification, oligonucleotides, which are coupled to fluorochromes and complementary to repeating sequences in the amplicons are used to detect sites of interaction. (Soderberg et al., 2006). In isPLA, the IC_{50} of MYCMI-6, MYCMI-11 and MYCMI-14 with respect to MYC:MAX interaction were found to be 1.5μM, 6μM and 6μM, respectively. The inhibitory effect of MYCMI-6 was measurable already after 3-hour treatment. U2OS cells containing MYC-ER was used to evaluate the effect of the compounds on MYC target gene expression. MYCMI-6 significantly repressed three selected MYC target genes (*ODC1, RSG16 and CR2*), while MYCMI-11 and MYCMI-14 were less efficient. Next, to examine whether MYCMI-6 targeted MYC:MAX directly, we performed biophysical interaction assays with recombinant bHLHZip domain of the MYC and MAX proteins. Here we first used microscale thermophoresis (MST), which is based on thermophoresis; the ability of fluorescent molecules to move in a temperature gradient, which will be affected by binding of ligands. Pretreatment with the three compounds perturbed the interaction between MYC and fluorophore-labeled MAX, and titration of MYCMI-6 showed a thermophoresis shift of K_d=4.3±2.9 μM for MYC:MAX, having only minor effect on MAX:MAX. In addition, surface plasmon resonance (SPR) was also performed to determine the affinity and monitor kinetics between protein and ligand. In SPR, the protein of interest is immobilized on a chip,

and the ligand of interest will flow over the chip surface. Upon binding of a ligand (or inhibitor) to the immobilized protein, changes will induced in the surface plasmon resonance , which can be measured (Schasfoort and Tudos, 2008). In this case, MYC or MAX was immobilized on a sensor chip. To explore the inhibition of MYC:MAX by MYCMI-6, the MAX bHLHZip was immobilized on the chip, and the MYC bHLHZip pretreated with or without MYCMI-6 was injected and allowed to flow over the chip. The IC_{50} of MYCMI-6 inhibition of MYC:MAX interaction was 3.8±1.2 μM, which was much more potent compared with the experimental MYC-MAX inhibitors 10058-F4 and KJ-Pyr-9 used as references.

We next wanted to know if MYCMI-6 binds directly to MYC or MAX. By using the recombinant bHLHZip domain of MYC or MAX alone in the MST assay, we confirmed that MYCMI-6 bound to MYC, rather than to MAX, in the low micromolar range. This high affinity was also validated in SPR assay with immobilized MYC on chip. MYCMI-6 was shown to bind with a K_D of 1.6±0.5 μM, in which K_D of another two known MYC:MAX inhibitors 10074-G5 and #474 were 28 μM and 15 μM, respectively. MYCMI-6 binding to a number of other proteins, MAX, MXD1, p53 and BSA was negligible, and MYCMI-6 at 2 μM only had 10% of the theoretical maximum to BCL-X_L. Thus, MYCMI-6 directly and selectively bound to MYC bHLHZip domain with high affinity.

We next evaluated whether MYCMI-6 could inhibit tumor cell growth and whether this correlated with MYC level. MYCMI-6 displayed a high efficacy to suppress the growth of MYC-driven tumor cells, e.g. neuroblastoma cells (GI_{50} ~2.5-6 μM) and Burkitt's lymphoma (GI_{50}= 0.5 μM) cells *in vitro*. MYCMI-6-mediated MYCN:MAX interruption was confirmed by decreased MYCN:MAX signals in isPLA and by suppression of MYCN target genes and anchorage-independent growth in *MYCN*-amplifed neuroblastoma cells. Moreover, we also found a strong correlation between MYC expression level and growth inhibitory response in NCI-60 tumor cell lines, in which cell lines with 'higher MYC' have significantly more probability to respond MYCMI-6 treatment than cells with 'lower MYC'. Importantly, MYCMI-6 was not cytotoxic to normal human cells. In addition, MYCMI-6 reduced *MYCN*-amplified neuroblastoma cell proliferation in a xenograft tumor model *in vivo*, partially resulting from MYCMI-6-triggered apoptosis. Data from isPLA also displayed a reduction of MYC:MAX interaction upon MYCMI-6 treatment *in vivo*. Therefore, MYCMI-6 suppressed MYC function in MYC-driven tumor cells via selective disruption of MYC:MAX interaction, leading to cell proliferation arrest and apoptosis.

In terms of MYCMI-7, we also confirmed inhibition of endogenous MYC:MAX interaction using the isPLA assay, and co-immunoprecipitation assay showed that this interruption started already within an 1 hour of treatment, suggesting a prompt inhibition of MYC:MAX interaction mediated by MYCMI-7, without affecting MYC protein level at early time points. Chromatin immunoprecipitation (ChIP) assay also revealed that MYCMI-7 led to dissociation of MYC from promoters of MYC target genes starting already at 2 hours post treatment. Interrupting MYC:MAX also led to repression of the MYC target gene *CR2* in

U2OS-MycER system. Using recombinant bHLHZip domain of MYC and MAX in SPR also indicated that MYCMI-7 inhibited MYC:MAX interaction directly and bound to MYC with an affinity of approximately 4 μM.

We next addressed the mechanism of the reduced endogenous MYC expression observed after prolonged treatment with MYCMI-7. Our results showed that MYCMI-7 decreased the MYC protein level but did not affect the mRNA expression. While MYCMI-7 increased MYC protein turnover somewhat in HCT116 cells it did not seem to affect the turnover rate in Hela cells, suggesting that this is cell-context-dependent. FBW7 is known to induce degradation of MYC by binding Thr 58-phosphorylated MYC, but phosphorylation of Thr 58 or Ser 62 was not been altered, and MYC T58A and S62A mutants were still reduced in expression after upon MYCMI-7 treatment, which argues that FBXW7 is not directly involved in MYCMI-7 action. Another possibility is that MYCMI-7 affects the MYC mRNA translation process, which requires further investigation.

We next studied if MYCMI-7 affected cell growth in a MYC-dependent manner. For this purpose, we used in Rat1 fibroblasts with different MYC status (wild-type MYC, MYC null and reconstituted MYC), Both MYCMI-6 and MYCMI-7 inhibited growth of wt and MYC-reconstituted cells, but not MYC null cells, thus showing that the compounds inhibited growth in a MYC-dependent manner. Similar to MYCMI-6, MYCMI-7 preferentially inhibited growth of *MYCN*-amplified compared with non-amplified neuroblastoma cells. It also strongly inhibited growth of Burkitt's lymphoma cells with MYC translocation, and a good correlation was found between MYCMI-7 inhibition and MYC expression level in NCI-60 human tumor cell line panel. Further, while MYCMI-7 only had a cytostatic effect on normal cells, it triggered apoptosis in tumor cells, thereby showing a good therapeutic window. Using an *ex vivo* cell panel derived from primary glioblastoma tumor biopsies from 42 patients, MYCMI-7 induced growth inhibition with an EC_{50} in the submicromolar range, and growth inhibition was also observed in primary acute myeloid leukemia (AML) tumor biopsies. In addition, MYCMI-7 also reduced tumor burden in an MYC-driven AML allogeneic mouse model. Further, in a basal-like breast cancer xenograft model and *MYCN*-amplified neuroblastoma xenograft model, besides tumor regression, MYCMI-7 also prolonged survival. In summary, MYCMI-7 inhibits MYC:MAX inhibition with high efficacy in cells and *in vitro*, binds recombinant MYC directly at low mM concentrations, downregulates MYC protein expression, inhibits MYC-driven tumor cell growth *in vitro* and *in vivo* resulting in reduced tumor burden and increased survival *in vivo*.

The comparison between MYCMI-6 and MYCMI-7 indicated different mechanisms probably mediated by two MYC:MAX inhibitors. Unlike MYCMI-6, MYCMI-7 not only inhibited MYC:MAX interaction, but also decreased steady state of MYC level in different cell lines. The reason for this difference is unclear. Though both of them target the MYC bHLHZip domain, the precise binding sites may be different. This may for instance affect the folding of the proteins differently, which may affect how the proteins are targeted for proteolysis. It is also possible that either compound affects the interaction between MYC and also other

MYC-binding proteins, which also may have different outcomes. Another possibility would be that the compounds also have other targets other than MYC:MAX. All these require further investigation.

3.4 PAPER V: MYC INHIBITORS WORK SYNERGISTICALLY WITH BRAF, MAPK AND CDK INHIBITORS TO BLOCK MALIGNANT MELANOMA CELL GROWTH BY INDUCTION OF CELLULAR SENESCENCE

MYC expression is commonly deregulated in cancer and often linked to aggressive disease, including in melanoma (Beroukhim et al., 2010; Ciriello et al., 2013), which makes MYC as a prioritized target for anti-cancer therapy. However, there are no specific MYC inhibitors available in clinic. This motivated us to pursue a screening campaign for more efficient and selective MYC inhibitors, leading to the identification of MYCMI-6 (**Paper III)** (Castell et al., 2018) and MYCMI-7 (**Paper IV)**. MYC inhibition is reported to induce senescence in melanoma cells with BRAF/NRAS mutation (Zhuang et al., 2008). In **Paper V**, we explored the potential of the MYC:MAX inhibitor MYCMI-7, as well as other experimental MYC inhibitors, including two MYC:MAX inhibitors10058-F4 (F4) and MYCMI-6, a BET inhibitor JQ1, which represses *MYC* transcription in certain cells, to induce senescence in melanoma cells as potential pro-senescence therapy.

First, we performed a senescence screen using MYCMI-7 and F4, and the same panel of melanoma cells as in **Paper II**. Compared to F4, MYCMI-7 displayed a more potent growth inhibitory effect and induced signs of senescence in the majority of cell lines, preferentially those with $BRAF^{V600E}$ mutation. In addition, MYCMI-7 also enhanced HLA class I expression at higher doses. Senescence induction was further validated using selected cell lines with $BRAF^{V600E}$ mutation and three MYC inhibitors, MYCMI-7, F4 and JQ1. We found that the different MYC inhibitors exerted different effects in terms of senescence and apoptosis induction. For example, in A375 cells, F4 was a potent trigger of DNA damage and apoptosis 24 hrs post treatment without activating p53 or p21, while MYCMI-7 induced p53 activation already at lower dose as well as p21, indicative of senescence induction, but caused apoptosis only at higher dose. In contrast, JQ1 triggered high expression of p21 without induction of apoptosis. Interestingly, unlike F4 and JQ1, MYCMI-7 suppressed cell growth persistently after drug removal. The $BRAF^{V600E}$ inhibitor vemurafenib and the MEK inhibitor trametinib are used clinically for melanoma treatment, but patients eventually develop resistance to these treatments. In order to evaluate if the MYC inhibitors influenced vemurafenib/trametinib resistance, a senescence screen was performed using BRAF or MEK inhibitors in combination with MYC inhibitors. The results showed that the MYC inhibitors synergized with BRAF inhibitor/MEK inhibitors to induce senescence and overcome vemurafenib-induced resistance in most cases. In **Paper II**, we found the CDK4/6 inhibitor palbociclib induced senescence in most melanoma cell lines, irrespective of BRAF/NRAS mutations. We therefore explored the potential of combining palbociclib and MYC inhibitor treatments to overcome vemurafenib-induced resistance. The results showed that the

combined treatment displayed a synergistic effect in inducing senescence in melanoma cells, also in vemurafenib-resistant cells. In addition, combinations of the MYC inhibitors with the traditional DNA alkylating agent temozolomide or the experimental p53:MDM2 inhibitor RITA were also effective in some but not all cell lines.

Our results confirmed a previous publication by Zhuang et al. that siRNA-mediated depletion of MYC triggered senescence in melanoma cells (Zhuang et al., 2008), and extended this to experimental pharmacological MYC inhibitors. However, we also observed some differences in effects among the different pharmacological MYC inhibitors. For example, at least in A375 cells, F4 seemed favor apoptosis over senescence, MYCMI-7 treatment resulted in a mix of senescent and apoptotic cells, while JQ1 preferentially induced signs of senescence. However, only MYCMI-7 induced a persistent growth inhibitory effect compared with F4 and JQ1. One explanation for these differences could be that JQ1 targets MYC transcription, while the other compounds inhibit the MYC:MAX interaction but also downregulates MYC protein levels, presumably through different mechanisms (**Paper II** (Castell et al., 2018) and **Paper III**). Moreover, F4 and MYCMI-7 treatment both induced DNA damage, which could trigger both apoptosis and senescence dependent on cell context, while JQ1 did not. Since JQ1 is a BET inhibitor, it is likely to affect a number of other genes apart from MYC, which may also contribute to the outcome. Also, one cannot exclude that F4 and MYCMI-7 have other targets as well.

Interestingly, palbociclib showed a good synergistic effect with the MYC inhibitors, including MYCMI-6, with respect to senescence induction, which potentially could provide an alternative treatment strategy in the future in drug-resistant melanoma. CDK4 is a well-known MYC target gene (Hermeking et al., 2000) and the oncogenic function CDK4 and MYC were mutually dependent on each other for malignant transformation (Haas et al., 1997; Miliani de Marval et al., 2004), indicating a close cooperation between CDK4 and MYC. Therefore, targeting these two factors should have synergistic effects, as shown here. Moreover, there are implications about the role of MYC in the temozolomide and RITA treatment from previous research. Regarding the synergism between MYC and temozolomide, temozolomide has been suggested to inhibit human glioblastoma via suppression of MYC and activation of TAp63 (Yamaki et al., 2013). RITA and MYC inhibitors did show a good dose-dependent senescence induction in A375-VR4 cells with synergistic effects. Given that A375-VR4 cells have wild type of p53, RITA-mediated senescence may work through reactivation of p53, alternatively by triggering the DNA damage response, which in turn activate p53 as an effector (Ahmed et al., 2011; de Lange et al., 2012), leading to senescence induction. This may partially overlap with mechanisms of F4/MYCMI-7-induced senescence, resulting in synergism. Further, dual treatment of RITA and another BET inhibitor CPI-203 synergized chronic myeloid leukemia (CML) undergoing cell death, differentiation and elimination of leukaemic stem cells (Abraham et al., 2016). Thus, the combination of palbociclib, temozolomide or RITA with MYCi may improve treatment for drug-resistant melanoma.

Moreover, one important feature of senescence is SASP, and therefore it would be intriguing to examine the expression of SASP and additional immune markers both in vitro and *in vivo* upon MYC inhibition, to uncover the potential of anti-MYC pro-senescence therapy with immunosurveillance activation. Intriguingly, it has been confirmed that both *CD47* and *PD-L1* are MYC target genes, leading to immune evasion of tumor cells (Casey et al., 2017; Casey et al., 2016; Xu et al., 2019). These could be downregulated upon JQ1 treatment and result in immune stimulation and tumor regression (Casey et al., 2016; Zhu et al., 2016a). In addition, Kortlever et al. also reported that MYC overexpression led to recruitment of macrophages, expelled T and B cells and favored angiogenesis due to secretion of CCL9 and IL-23, and PD-L1 induction, eventually switching $KRAS^{G12D}$-driven adenomas into proliferative and invasive adenocarcinomas (Kortlever et al., 2017). Thus, targeting MYC with other factors can be a win-win opportunity to both arrest cell proliferation and re-establish immunosurveillance.

4 ACKNOWLEDGEMENTS

First and foremost, I would like to thank my main supervisor **Lars-Gunnar Larsson**, for your great supervision and encouragement persistently during my whole PhD studies. You are definitely the most dedicate and passionate to science, which always motivates me to pursue my goal, not only in academia, but also in my life. I will be forever grateful for your pedagogic explanation for my confusion, your comments for nearly all my writing and your support for my wild guess. And I will never forget your iconic laugh and your passion in life! Thank you so much, Lars-Gunnar!

To my co-supervisor, **Alina Castell**, I would like to thank for all your patience and help when I have difficulties. Your excellent pedagogical way in discussion is always informative and inspired. I am lucky to have you as co-supervisor.

To my former co-supervisors. **Rolf Ohlsson**, thank you for sharing your idea and your laboratory resource with me. **Linda**, thank you for the supervision at the beginning of my studies, which was short but useful. **Jacob Goodwin**, you always brought new ideas and new methodologies to us. I think you are the pioneer in the lab and I was always willing to discuss with you. I really appreciate your supervision and your support at any time!

To my mentor, **Lifu Hu**. Thank you for caring about my career and future, as well as all invitations of career talks from different organizers, which broadens my horizon and provides more opportunities for my future.

To all members in Lars-Gunnar's lab. **Qinzi**, you are always the angel when I encounter difficulties at work and personal life. **Marcela**, I am glad to work with you because of your dedication to work and optimism to life. **Wesam**, what a coincidence that we met in Uppsala before and work in the same group in Stockholm now. Thank you for all the help for such long time of acquaintance. **Vicky**, both your expertise and your commitment amazed and inspired me. Thank you for all the help and discussion! I really missed the pleasant period of working with you. **Nyosha**, I am impressed by your independence and your dedication and thank you for being supportive for me all the time! **Mariam**, thank you for all your help and advice for projects and personal life. **Hamid**, you are definitely the ChIP master and you are the kindest person in my eyes! **Per**, I was impressed by your professional and great pleasure to have the chance to work with you! **Sara**, you prove to me how efficient a person could be. **Sanna**, I was amazed by your persistence and belief in projects and your caring to your family at the meantime. **Tamer**, nice to work with you and your questions always improve my understanding of my project. **Samudra**, you are so diligent and enthusiastic to your work, and I believe you will achieve your goal in academia soon!

Thanks to all the collaborators! In particular, I would like to thank **Galina** and **Madhu** for kind effort and support during twice collaboration. Moreover, I also appreciate the full support in the melanoma consortium for Wallenberg founding: **Ishani Das**, **Sofi**, **Jeroen**, **Marina**, **Alireza**, **Michelle**, **Rainer**, **Fredrik**, **Suzanne**, **Johan**, **Rolf**, **Margareta** and **Klas**.

I also would like to thank people who have offered me in MTC: **Galina's group**: **Sylvain**, **Ali**, **Jiawei** and **Xiaolei**; **Sonia's group**: **Marcus**, **Ingeborg**, **Tanzina** and **Gergana**; **Marie's** and **Margareta's** group: **Ganna**, **Aida**, **Maria**, **Lourdes, Johanna, Ann and Evelyn**; **Yihai's group**: **Kayoko**, **Junwei**, **Jieyu**, **Xingkang**; **Ingemar's group**: **Shixing**; **Gerald's group**: **Lifeng**, **Marc** and **Ben**.

Moreover, I would like to thank all my buddies: Chinese best neighbors: **Lei Li** and **Xiaogang Feng**; Chinese best classmates: **Shengze Yu**, **Zhe Zhang**, **Hanyu Qin** and **Jing Xu**; Uppsala best roommates: **Yilin Wang**, **Bowen Liu** and **Airy**; PS&X-Box group: **Dongmei Tong**, **Tenghao Zheng**, **Dan Dou** and **Hao Wang**. Friends in MTC: **Honglei Zhao**, **Yu Gao** and **Srisuda** and my former supervisor: **Antonia**!

I also have to say thank you to my **fluffy kitties**, for your accompany (sleeping) while I was writing thesis; for your waiting for me (food) when I came back late; for your caring (standing, scratching or sleeping) for my computer! All of you bring colors and happiness into my boring life! Let's stay together and keep healthy! 😈

Last but definitely not the least, thank **my families** for several years' support and understanding and thank my boyfriend **Fan Yang** for sharing, trusting, encouraging and cooking!

5 REFERENCES

Abraham, S.A., Hopcroft, L.E., Carrick, E., Drotar, M.E., Dunn, K., Williamson, A.J., Korfi, K., Baquero, P., Park, L.E., Scott, M.T.*, et al.* (2016). Dual targeting of p53 and c-MYC selectively eliminates leukaemic stem cells. Nature *534*, 341-346.

Acosta, J.C., and Gil, J. (2012). Senescence: a new weapon for cancer therapy. Trends Cell Biol *22*, 211-219.

Acosta, J.C., O'Loghlen, A., Banito, A., Guijarro, M.V., Augert, A., Raguz, S., Fumagalli, M., Da Costa, M., Brown, C., Popov, N.*, et al.* (2008). Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell *133*, 1006-1018.

Adachi, M., Roussel, M.F., Havenith, K., and Sherr, C.J. (1997). Features of macrophage differentiation induced by p19INK4d, a specific inhibitor of cyclin D-dependent kinases. Blood *90*, 126-137.

Adams, M.R., Sears, R., Nuckolls, F., Leone, G., and Nevins, J.R. (2000). Complex transcriptional regulatory mechanisms control expression of the E2F3 locus. Mol Cell Biol *20*, 3633-3639.

Adhikary, S., Marinoni, F., Hock, A., Hulleman, E., Popov, N., Beier, R., Bernard, S., Quarto, M., Capra, M., Goettig, S.*, et al.* (2005). The ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proliferation. Cell *123*, 409-421.

Ahmed, A., Yang, J., Maya-Mendoza, A., Jackson, D.A., and Ashcroft, M. (2011). Pharmacological activation of a novel p53-dependent S-phase checkpoint involving CHK-1. Cell Death Dis *2*, e160.

Aird, K.M., Zhang, G., Li, H., Tu, Z., Bitler, B.G., Garipov, A., Wu, H., Wei, Z., Wagner, S.N., Herlyn, M.*, et al.* (2013). Suppression of nucleotide metabolism underlies the establishment and maintenance of oncogene-induced senescence. Cell Rep *3*, 1252-1265.

Arabi, A., Wu, S., Ridderstrale, K., Bierhoff, H., Shiue, C., Fatyol, K., Fahlen, S., Hydbring, P., Soderberg, O., Grummt, I.*, et al.* (2005). c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. Nat Cell Biol *7*, 303-310.

Arango Duque, G., and Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. Front Immunol *5*, 491.

Arnold, H.K., Zhang, X., Daniel, C.J., Tibbitts, D., Escamilla-Powers, J., Farrell, A., Tokarz, S., Morgan, C., and Sears, R.C. (2009). The Axin1 scaffold protein promotes formation of a degradation complex for c-Myc. EMBO J *28*, 500-512.

Atsaves, V., Tsesmetzis, N., Chioureas, D., Kis, L., Leventaki, V., Drakos, E., Panaretakis, T., Grander, D., Medeiros, L.J., Young, K.H.*, et al.* (2017). PD-L1 is commonly expressed and transcriptionally regulated by STAT3 and MYC in ALK-negative anaplastic large-cell lymphoma. Leukemia *31*, 1633-1637.

Bahram, F., Hydbring, P., Tronnersjo, S., Zakaria, S.M., Frings, O., Fahlen, S., Nilsson, H., Goodwin, J., von der Lehr, N., Su, Y.*, et al.* (2016). Interferon-gamma-induced p27KIP1 binds to and targets MYC for proteasome-mediated degradation. Oncotarget *7*, 2837-2854.

Baker, D.J., and Sedivy, J.M. (2013). Probing the depths of cellular senescence. J Cell Biol *202*, 11-13.

Bannister, A.J., and Kouzarides, T. (2011). Regulation of chromatin by histone modifications. Cell Res *21*, 381-395.

Benassi, B., Fanciulli, M., Fiorentino, F., Porrello, A., Chiorino, G., Loda, M., Zupi, G., and Biroccio, A. (2006). c-Myc phosphorylation is required for cellular response to oxidative stress. Mol Cell *21*, 509-519.

Berg, T., Cohen, S.B., Desharnais, J., Sonderegger, C., Maslyar, D.J., Goldberg, J., Boger, D.L., and Vogt, P.K. (2002). Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. P Natl Acad Sci USA *99*, 3830-3835.

Beroukhim, R., Mermel, C.H., Porter, D., Wei, G., Raychaudhuri, S., Donovan, J., Barretina, J., Boehm, J.S., Dobson, J., Urashima, M.*, et al.* (2010). The landscape of somatic copy-number alteration across human cancers. Nature *463*, 899-905.

Beurel, E., Grieco, S.F., and Jope, R.S. (2015). Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol Ther *148*, 114-131.

Beyne-Rauzy, O., Recher, C., Dastugue, N., Demur, C., Pottier, G., Laurent, G., Sabatier, L., and Mansat-De Mas, V. (2004). Tumor necrosis factor alpha induces senescence and chromosomal instability in human leukemic cells. Oncogene *23*, 7507-7516.

Bhadury, J., Nilsson, L.M., Muralidharan, S.V., Green, L.C., Li, Z., Gesner, E.M., Hansen, H.C., Keller, U.B., McLure, K.G., and Nilsson, J.A. (2014). BET and HDAC inhibitors induce similar genes and biological effects and synergize to kill in Myc-induced murine lymphoma. Proc Natl Acad Sci U S A *111*, E2721-2730.

Bhat, M., Robichaud, N., Hulea, L., Sonenberg, N., Pelletier, J., and Topisirovic, I. (2015). Targeting the translation machinery in cancer. Nat Rev Drug Discov *14*, 261-278.

Binne, U.K., Classon, M.K., Dick, F.A., Wei, W., Rape, M., Kaelin, W.G., Jr., Naar, A.M., and Dyson, N.J. (2007). Retinoblastoma protein and anaphase-promoting complex physically interact and functionally cooperate during cell-cycle exit. Nat Cell Biol *9*, 225-232.

Bister, K., Hayman, M.J., and Vogt, P.K. (1977). Defectiveness of avian myelocytomatosis virus MC29: isolation of long-term nonproducer cultures and analysis of virus-specific polypeptide synthesis. Virology *82*, 431-448.

Blackwell, T.K., Kretzner, L., Blackwood, E.M., Eisenman, R.N., and Weintraub, H. (1990). Sequence-specific DNA binding by the c-Myc protein. Science *250*, 1149-1151.

Blackwood, E.M., and Eisenman, R.N. (1991). Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science *251*, 1211-1217.

Blackwood, E.M., Luscher, B., and Eisenman, R.N. (1992). Myc and Max associate in vivo. Genes Dev *6*, 71-80.

Bouchard, C., Marquardt, J., Bras, A., Medema, R.H., and Eilers, M. (2004). Myc-induced proliferation and transformation require Akt-mediated phosphorylation of FoxO proteins. EMBO J *23*, 2830-2840.

Boxer, R.B., Jang, J.W., Sintasath, L., and Chodosh, L.A. (2004). Lack of sustained regression of c-MYC-induced mammary adenocarcinomas following brief or prolonged MYC inactivation. Cancer Cell *6*, 577-586.

Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A.H., Schlegelberger, B., Stein, H., Dorken, B., Jenuwein, T., and Schmitt, C.A. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. Nature *436*, 660-665.

Brenner, C., Deplus, R., Didelot, C., Loriot, A., Vire, E., De Smet, C., Gutierrez, A., Danovi, D., Bernard, D., Boon, T.*, et al.* (2005). Myc represses transcription through recruitment of DNA methyltransferase corepressor. EMBO J *24*, 336-346.

Brodeur, G.M. (2003). Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer *3*, 203-216.

Campaner, S., Doni, M., Hydbring, P., Verrecchia, A., Bianchi, L., Sardella, D., Schleker, T., Perna, D., Tronnersjo, S., Murga, M.*, et al.* (2010). Cdk2 suppresses cellular senescence induced by the c-myc oncogene. Nat Cell Biol *12*, 54-59; sup pp 51-14.

Cao, F., Fang, Y., Tan, H.K., Goh, Y., Choy, J.Y.H., Koh, B.T.H., Hao Tan, J., Bertin, N., Ramadass, A., Hunter, E.*, et al.* (2017). Super-Enhancers and Broad H3K4me3 Domains Form Complex Gene Regulatory Circuits Involving Chromatin Interactions. Sci Rep *7*, 2186.

Casey, S.C., Baylot, V., and Felsher, D.W. (2017). MYC: Master Regulator of Immune Privilege. Trends Immunol *38*, 298-305.

Casey, S.C., Tong, L., Li, Y., Do, R., Walz, S., Fitzgerald, K.N., Gouw, A.M., Baylot, V., Gutgemann, I., Eilers, M.*, et al.* (2016). MYC regulates the antitumor immune response through CD47 and PD-L1. Science *352*, 227-231.

Castell, A., and Larsson, L.G. (2015). Targeting MYC Translation in Colorectal Cancer. Cancer Discov *5*, 701-703.

Castell, A., Yan, Q., Fawkner, K., Hydbring, P., Zhang, F., Verschut, V., Franco, M., Zakaria, S.M., Bazzar, W., Goodwin, J.*, et al.* (2018). A selective high affinity MYC-binding compound inhibits MYC:MAX interaction and MYC-dependent tumor cell proliferation. Sci Rep *8*, 10064.

Cepeda, D., Ng, H.F., Sharifi, H.R., Mahmoudi, S., Cerrato, V.S., Fredlund, E., Magnusson, K., Nilsson, H., Malyukova, A., Rantala, J.*, et al.* (2013). CDK-mediated activation of the SCF(FBXO) (28) ubiquitin ligase promotes MYC-driven transcription and tumourigenesis and predicts poor survival in breast cancer. EMBO Mol Med *5*, 1067-1086.

Chaidos, A., Caputo, V., Gouvedenou, K., Liu, B., Marigo, I., Chaudhry, M.S., Rotolo, A., Tough, D.F., Smithers, N.N., Bassil, A.K.*, et al.* (2014). Potent antimyeloma activity of the novel bromodomain inhibitors I-BET151 and I-BET762. Blood *123*, 697-705.

Chandra, T., Ewels, P.A., Schoenfelder, S., Furlan-Magaril, M., Wingett, S.W., Kirschner, K., Thuret, J.Y., Andrews, S., Fraser, P., and Reik, W. (2015). Global reorganization of the nuclear landscape in senescent cells. Cell Rep *10*, 471-483.

Chandra, T., Kirschner, K., Thuret, J.Y., Pope, B.D., Ryba, T., Newman, S., Ahmed, K., Samarajiwa, S.A., Salama, R., Carroll, T.*, et al.* (2012). Independence of repressive histone marks and chromatin compaction during senescent heterochromatic layer formation. Mol Cell *47*, 203-214.

Chang, J., Wang, Y., Shao, L., Laberge, R.M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N.E., Ding, S., Feng, W.*, et al.* (2016). Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med *22*, 78-83.

Chen, Z., Trotman, L.C., Shaffer, D., Lin, H.K., Dotan, Z.A., Niki, M., Koutcher, J.A., Scher, H.I., Ludwig, T., Gerald, W.*, et al.* (2005). Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature *436*, 725-730.

Cheng, S.W.G., Davies, K.P., Yung, E., Beltran, R.J., Yu, J., and Kalpana, G.V. (1999). c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. Nature Genetics *22*, 102-105.

Chicas, A., Wang, X., Zhang, C., McCurrach, M., Zhao, Z., Mert, O., Dickins, R.A., Narita, M., Zhang, M., and Lowe, S.W. (2010). Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. Cancer Cell *17*, 376-387.

Chiche, A., Le Roux, I., von Joest, M., Sakai, H., Aguin, S.B., Cazin, C., Salam, R., Fiette, L., Alegria, O., Flamant, P.*, et al.* (2017). Injury-Induced Senescence Enables In Vivo Reprogramming in Skeletal Muscle. Cell Stem Cell *20*, 407-414 e404.

Chien, Y., Scuoppo, C., Wang, X., Fang, X., Balgley, B., Bolden, J.E., Premsrirut, P., Luo, W., Chicas, A., Lee, C.S.*, et al.* (2011). Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. Genes Dev *25*, 2125-2136.

Childs, B.G., Durik, M., Baker, D.J., and van Deursen, J.M. (2015). Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med *21*, 1424-1435.

Ciriello, G., Miller, M.L., Aksoy, B.A., Senbabaoglu, Y., Schultz, N., and Sander, C. (2013). Emerging landscape of oncogenic signatures across human cancers. Nat Genet *45*, 1127-1133.

Claassen, G.F., and Hann, S.R. (2000). A role for transcriptional repression of p21CIP1 by c-Myc in overcoming transforming growth factor beta -induced cell-cycle arrest. Proc Natl Acad Sci U S A *97*, 9498-9503.

Clevers, H. (2004). Wnt breakers in colon cancer. Cancer Cell *5*, 5-6.

Cole, M.D., and Cowling, V.H. (2008). Transcription-independent functions of MYC: regulation of translation and DNA replication. Nat Rev Mol Cell Biol *9*, 810-815.

Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A.J., Barradas, M., Benguria, A., Zaballos, A., Flores, J.M., Barbacid, M.*, et al.* (2005). Tumour biology: senescence in premalignant tumours. Nature *436*, 642.

Collado, M., and Serrano, M. (2010). Senescence in tumours: evidence from mice and humans. Nature reviews Cancer *10*, 51-57.

Coller, H.A., Grandori, C., Tamayo, P., Colbert, T., Lander, E.S., Eisenman, R.N., and Golub, T.R. (2000). Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. Proc Natl Acad Sci U S A *97*, 3260-3265.

Coppe, J.P., Desprez, P.Y., Krtolica, A., and Campisi, J. (2010). The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol *5*, 99-118.

Coppe, J.P., Kauser, K., Campisi, J., and Beausejour, C.M. (2006). Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem *281*, 29568-29574.

Coppe, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., and Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS biology *6*, 2853-2868.

Corthay, A. (2009). How do regulatory T cells work? Scand J Immunol *70*, 326-336.

Cottini, F., Hideshima, T., Suzuki, R., Tai, Y.T., Bianchini, G., Richardson, P.G., Anderson, K.C., and Tonon, G. (2015). Synthetic Lethal Approaches Exploiting DNA Damage in Aggressive Myeloma. Cancer Discov *5*, 972-987.

Cowling, V.H., Chandriani, S., Whitfield, M.L., and Cole, M.D. (2006). A conserved Myc protein domain, MBIV, regulates DNA binding, apoptosis, transformation, and G2 arrest. Mol Cell Biol *26*, 4226-4239.

Cowling, V.H., and Cole, M.D. (2006). Mechanism of transcriptional activation by the Myc oncoproteins. Semin Cancer Biol *16*, 242-252.

Cowling, V.H., and Cole, M.D. (2007). The Myc transactivation domain promotes global phosphorylation of the RNA polymerase II carboxy-terminal domain independently of direct DNA binding. Mol Cell Biol *27*, 2059-2073.

Criscione, S.W., De Cecco, M., Siranosian, B., Zhang, Y., Kreiling, J.A., Sedivy, J.M., and Neretti, N. (2016). Reorganization of chromosome architecture in replicative cellular senescence. Sci Adv *2*, e1500882.

Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M., and Hemmings, B.A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature *378*, 785-789.

D'Azzo, A., Hoogeveen, A., Reuser, A.J., Robinson, D., and Galjaard, H. (1982). Molecular defect in combined beta-galactosidase and neuraminidase deficiency in man. Proc Natl Acad Sci U S A *79*, 4535-4539.

Dang, C.V. (2010). Rethinking the Warburg effect with Myc micromanaging glutamine metabolism. Cancer Res *70*, 859-862.

Dang, C.V. (2012). MYC on the path to cancer. Cell *149*, 22-35.

Dang, C.V. (2013). MYC, metabolism, cell growth, and tumorigenesis. Cold Spring Harb Perspect Med *3*.

Das Thakur, M., Salangsang, F., Landman, A.S., Sellers, W.R., Pryer, N.K., Levesque, M.P., Dummer, R., McMahon, M., and Stuart, D.D. (2013). Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. Nature *494*, 251-255.

Dawson, M.A., Prinjha, R.K., Dittmann, A., Giotopoulos, G., Bantscheff, M., Chan, W.I., Robson, S.C., Chung, C.W., Hopf, C., Savitski, M.M*., et al.* (2011). Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature *478*, 529-533.

De Cecco, M., Criscione, S.W., Peckham, E.J., Hillenmeyer, S., Hamm, E.A., Manivannan, J., Peterson, A.L., Kreiling, J.A., Neretti, N., and Sedivy, J.M. (2013). Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. Aging Cell *12*, 247-256.

De Cecco, M., Jeyapalan, J., Zhao, X., Tamamori-Adachi, M., and Sedivy, J.M. (2011). Nuclear protein accumulation in cellular senescence and organismal aging revealed with a novel single-cell resolution fluorescence microscopy assay. Aging (Albany NY) *3*, 955-967.

de Lange, J., Verlaan-de Vries, M., Teunisse, A.F., and Jochemsen, A.G. (2012). Chk2 mediates RITA-induced apoptosis. Cell Death Differ *19*, 980-989.

de Visser, K.E., Eichten, A., and Coussens, L.M. (2006). Paradoxical roles of the immune system during cancer development. Nat Rev Cancer *6*, 24-37.

Dean, M., Levine, R.A., Ran, W., Kindy, M.S., Sonenshein, G.E., and Campisi, J. (1986). Regulation of c-myc transcription and mRNA abundance by serum growth factors and cell contact. J Biol Chem *261*, 9161-9166.

Delmore, J.E., Issa, G.C., Lemieux, M.E., Rahl, P.B., Shi, J., Jacobs, H.M., Kastritis, E., Gilpatrick, T., Paranal, R.M., Qi, J.*, et al.* (2011). BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell *146*, 904-917.

Demaria, M., Ohtani, N., Youssef, S.A., Rodier, F., Toussaint, W., Mitchell, J.R., Laberge, R.M., Vijg, J., Van Steeg, H., Dolle, M.E.*, et al.* (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell *31*, 722-733.

Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P.G., Bensimon, A.*, et al.* (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature *444*, 638-642.

Di Micco, R., Sulli, G., Dobreva, M., Liontos, M., Botrugno, O.A., Gargiulo, G., dal Zuffo, R., Matti, V., d'Ario, G., Montani, E.*, et al.* (2011). Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. Nat Cell Biol *13*, 292-302.

Di Mitri, D., Toso, A., Chen, J.J., Sarti, M., Pinton, S., Jost, T.R., D'Antuono, R., Montani, E., Garcia-Escudero, R., Guccini, I.*, et al.* (2014). Tumour-infiltrating Gr-1+ myeloid cells antagonize senescence in cancer. Nature *515*, 134-137.

Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O.*, et al.* (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A *92*, 9363-9367.

Dominguez-Sola, D., and Gautier, J. (2014). MYC and the control of DNA replication. Cold Spring Harb Perspect Med *4*.

Dominguez-Sola, D., Ying, C.Y., Grandori, C., Ruggiero, L., Chen, B., Li, M., Galloway, D.A., Gu, W., Gautier, J., and Dalla-Favera, R. (2007). Non-transcriptional control of DNA replication by c-Myc. Nature *448*, 445-451.

Donato, E., Croci, O., Sabo, A., Muller, H., Morelli, M.J., Pelizzola, M., and Campaner, S. (2017). Compensatory RNA polymerase 2 loading determines the efficacy and transcriptional selectivity of JQ1 in Myc-driven tumors. Leukemia *31*, 479-490.

Dorr, J.R., Yu, Y., Milanovic, M., Beuster, G., Zasada, C., Dabritz, J.H., Lisec, J., Lenze, D., Gerhardt, A., Schleicher, K.*, et al.* (2013). Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Nature *501*, 421-425.

Dou, Z., Ivanov, A., Adams, P.D., and Berger, S.L. (2016). Mammalian autophagy degrades nuclear constituents in response to tumorigenic stress. Autophagy *12*, 1416-1417.

Dou, Z., Xu, C., Donahue, G., Shimi, T., Pan, J.A., Zhu, J., Ivanov, A., Capell, B.C., Drake, A.M., Shah, P.P.*, et al.* (2015). Autophagy mediates degradation of nuclear lamina. Nature *527*, 105-109.

Dreesen, O., Chojnowski, A., Ong, P.F., Zhao, T.Y., Common, J.E., Lunny, D., Lane, E.B., Lee, S.J., Vardy, L.A., Stewart, C.L.*, et al.* (2013). Lamin B1 fluctuations have differential effects on cellular proliferation and senescence. J Cell Biol *200*, 605-617.

Duan, J., Duan, J., Zhang, Z., and Tong, T. (2005). Irreversible cellular senescence induced by prolonged exposure to H2O2 involves DNA-damage-and-repair genes and telomere shortening. Int J Biochem Cell Biol *37*, 1407-1420.

Duesberg, P.H., Bister, K., and Moscovici, C. (1979). Avian acute leukemia virus MC29: conserved and variable RNA sequences and recombination with helper virus. Virology *99*, 121-134.

Eberhardy, S.R., and Farnham, P.J. (2001). c-Myc mediates activation of the cad promoter via a post-RNA polymerase II recruitment mechanism. J Biol Chem *276*, 48562-48571.

Eberhardy, S.R., and Farnham, P.J. (2002). Myc recruits P-TEFb to mediate the final step in the transcriptional activation of the cad promoter. J Biol Chem *277*, 40156-40162.

Eggert, T., Wolter, K., Ji, J., Ma, C., Yevsa, T., Klotz, S., Medina-Echeverz, J., Longerich, T., Forgues, M., Reisinger, F.*, et al.* (2016). Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. Cancer Cell *30*, 533-547.

Eilers, M., Schirm, S., and Bishop, J.M. (1991). The MYC protein activates transcription of the alpha-prothymosin gene. EMBO J *10*, 133-141.

Eischen, C.M., Packham, G., Nip, J., Fee, B.E., Hiebert, S.W., Zambetti, G.P., and Cleveland, J.L. (2001). Bcl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. Oncogene *20*, 6983-6993.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. Toxicol Pathol *35*, 495-516.

Evan, G.I., Wyllie, A.H., Gilbert, C.S., Littlewood, T.D., Land, H., Brooks, M., Waters, C.M., Penn, L.Z., and Hancock, D.C. (1992). Induction of apoptosis in fibroblasts by c-myc protein. Cell *69*, 119-128.

Evangelou, K., Bartkova, J., Kotsinas, A., Pateras, I.S., Liontos, M., Velimezi, G., Kosar, M., Liloglou, T., Trougakos, I.P., Dyrskjot, L.*, et al.* (2013). The DNA damage checkpoint precedes activation of ARF in response to escalating oncogenic stress during tumorigenesis. Cell Death Differ *20*, 1485-1497.

Falchook, G.S., Lewis, K.D., Infante, J.R., Gordon, M.S., Vogelzang, N.J., DeMarini, D.J., Sun, P., Moy, C., Szabo, S.A., Roadcap, L.T.*, et al.* (2012). Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. Lancet Oncol *13*, 782-789.

Farrell, A.S., and Sears, R.C. (2014). MYC degradation. Cold Spring Harbor perspectives in medicine *4*.

Felsher, D.W. (2010). MYC Inactivation Elicits Oncogene Addiction through Both Tumor Cell-Intrinsic and Host-Dependent Mechanisms. Genes Cancer *1*, 597-604.

Fernandez, P.C., Frank, S.R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A., and Amati, B. (2003). Genomic targets of the human c-Myc protein. Genes Dev *17*, 1115-1129.

Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W.B., Fedorov, O., Morse, E.M., Keates, T., Hickman, T.T., Felletar, I.*, et al.* (2010). Selective inhibition of BET bromodomains. Nature *468*, 1067-1073.

Filippi-Chiela, E.C., Bueno e Silva, M.M., Thome, M.P., and Lenz, G. (2015). Single-cell analysis challenges the connection between autophagy and senescence induced by DNA damage. Autophagy *11*, 1099-1113.

Fletcher, S., and Prochownik, E.V. (2015). Small-molecule inhibitors of the Myc oncoprotein. Biochim Biophys Acta *1849*, 525-543.

Freund, A., Laberge, R.M., Demaria, M., and Campisi, J. (2012). Lamin B1 loss is a senescence-associated biomarker. Mol Biol Cell *23*, 2066-2075.

Freund, A., Patil, C.K., and Campisi, J. (2011). p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J *30*, 1536-1548.

Fridman, A.L., and Tainsky, M.A. (2008). Critical pathways in cellular senescence and immortalization revealed by gene expression profiling. Oncogene *27*, 5975-5987.

Futosi, K., Fodor, S., and Mocsai, A. (2013). Neutrophil cell surface receptors and their intracellular signal transduction pathways. Int Immunopharmacol *17*, 638-650.

Gao, P., Tchernyshyov, I., Chang, T.C., Lee, Y.S., Kita, K., Ochi, T., Zeller, K.I., De Marzo, A.M., Van Eyk, J.E., Mendell, J.T.*, et al.* (2009). c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature *458*, 762-765.

Georgakopoulou, E.A., Tsimaratou, K., Evangelou, K., Fernandez Marcos, P.J., Zoumpourlis, V., Trougakos, I.P., Kletsas, D., Bartek, J., Serrano, M., and Gorgoulis, V.G. (2013). Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryo-preserved and archival tissues. Aging (Albany NY) *5*, 37-50.

Georgilis, A., Klotz, S., Hanley, C.J., Herranz, N., Weirich, B., Morancho, B., Leote, A.C., D'Artista, L., Gallage, S., Seehawer, M.*, et al.* (2018). PTBP1-Mediated Alternative Splicing Regulates the Inflammatory Secretome and the Pro-tumorigenic Effects of Senescent Cells. Cancer Cell *34*, 85-102 e109.

Gius, D.R., Ezhevsky, S.A., Becker-Hapak, M., Nagahara, H., Wei, M.C., and Dowdy, S.F. (1999). Transduced p16INK4a peptides inhibit hypophosphorylation of the retinoblastoma protein and cell cycle progression prior to activation of Cdk2 complexes in late G1. Cancer Res *59*, 2577-2580.

Glass, C.K., Saijo, K., Winner, B., Marchetto, M.C., and Gage, F.H. (2010). Mechanisms underlying inflammation in neurodegeneration. Cell *140*, 918-934.

Goel, S., DeCristo, M.J., Watt, A.C., BrinJones, H., Sceneay, J., Li, B.B., Khan, N., Ubellacker, J.M., Xie, S., Metzger-Filho, O.*, et al.* (2017). CDK4/6 inhibition triggers anti-tumour immunity. Nature *548*, 471-475.

Gomes, L.C., Di Benedetto, G., and Scorrano, L. (2011). During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat Cell Biol *13*, 589-598.

Gomez-Roman, N., Grandori, C., Eisenman, R.N., and White, R.J. (2003). Direct activation of RNA polymerase III transcription by c-Myc. Nature *421*, 290-294.

Gorgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., Venere, M., Ditullio, R.A., Jr., Kastrinakis, N.G., Levy, B.*, et al.* (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature *434*, 907-913.

Grandori, C., Gomez-Roman, N., Felton-Edkins, Z.A., Ngouenet, C., Galloway, D.A., Eisenman, R.N., and White, R.J. (2005). c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. Nat Cell Biol *7*, 311-318.

Grandori, C., Wu, K.J., Fernandez, P., Ngouenet, C., Grim, J., Clurman, B.E., Moser, M.J., Oshima, J., Russell, D.W., Swisshelm, K.*, et al.* (2003). Werner syndrome protein limits MYC-induced cellular senescence. Genes Dev *17*, 1569-1574.

Gregory, M.A., Qi, Y., and Hann, S.R. (2003). Phosphorylation by glycogen synthase kinase-3 controls c-myc proteolysis and subnuclear localization. J Biol Chem *278*, 51606-51612.

Grewal, S.I., and Moazed, D. (2003). Heterochromatin and epigenetic control of gene expression. Science *301*, 798-802.

Grinberg, A.V., Hu, C.D., and Kerppola, T.K. (2004). Visualization of Myc/Max/Mad family dimers and the competition for dimerization in living cells. Mol Cell Biol *24*, 4294-4308.

Grivennikov, S.I., Greten, F.R., and Karin, M. (2010). Immunity, inflammation, and cancer. Cell *140*, 883-899.

Haas, K., Staller, P., Geisen, C., Bartek, J., Eilers, M., and Moroy, T. (1997). Mutual requirement of CDK4 and Myc in malignant transformation: evidence for cyclin D1/CDK4 and p16INK4A as upstream regulators of Myc. Oncogene *15*, 179-192.

Hall, M., Bates, S., and Peters, G. (1995). Evidence for different modes of action of cyclin-dependent kinase inhibitors: p15 and p16 bind to kinases, p21 and p27 bind to cyclins. Oncogene *11*, 1581-1588.

Han, X., Tai, H., Wang, X., Wang, Z., Zhou, J., Wei, X., Ding, Y., Gong, H., Mo, C., Zhang, J.*, et al.* (2016). AMPK activation protects cells from oxidative stress-induced senescence via autophagic flux restoration and intracellular NAD(+) elevation. Aging Cell *15*, 416-427.

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. J Gerontol *11*, 298-300.

Harper, J.W., Elledge, S.J., Keyomarsi, K., Dynlacht, B., Tsai, L.H., Zhang, P., Dobrowolski, S., Bai, C., Connell-Crowley, L., Swindell, E.*, et al.* (1995). Inhibition of cyclin-dependent kinases by p21. Mol Biol Cell *6*, 387-400.

Henriksson, M., Selivanova, G., Lindstrom, M., and Wiman, K.G. (2001). Inactivation of Myc-induced p53-dependent apoptosis in human tumors. Apoptosis *6*, 133-137.

Hermeking, H., Rago, C., Schuhmacher, M., Li, Q., Barrett, J.F., Obaya, A.J., O'Connell, B.C., Mateyak, M.K., Tam, W., Kohlhuber, F.*, et al.* (2000). Identification of CDK4 as a target of c-MYC. Proc Natl Acad Sci U S A *97*, 2229-2234.

Hernandez-Segura, A., de Jong, T.V., Melov, S., Guryev, V., Campisi, J., and Demaria, M. (2017). Unmasking Transcriptional Heterogeneity in Senescent Cells. Curr Biol *27*, 2652-2660 e2654.

Herranz, N., Gallage, S., Mellone, M., Wuestefeld, T., Klotz, S., Hanley, C.J., Raguz, S., Acosta, J.C., Innes, A.J., Banito, A.*, et al.* (2015). mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat Cell Biol *17*, 1205-1217.

Hildebrand, D.G., Lehle, S., Borst, A., Haferkamp, S., Essmann, F., and Schulze-Osthoff, K. (2013). alpha-Fucosidase as a novel convenient biomarker for cellular senescence. Cell Cycle *12*, 1922-1927.

Hirai, H., Roussel, M.F., Kato, J.Y., Ashmun, R.A., and Sherr, C.J. (1995). Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. Mol Cell Biol *15*, 2672-2681.

Horn, H.F., and Vousden, K.H. (2007). Coping with stress: multiple ways to activate p53. Oncogene *26*, 1306-1316.

Hu-Lieskovan, S., Mok, S., Homet Moreno, B., Tsoi, J., Robert, L., Goedert, L., Pinheiro, E.M., Koya, R.C., Graeber, T.G., Comin-Anduix, B.*, et al.* (2015). Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med *7*, 279ra241.

Hu, C.D., Chinenov, Y., and Kerppola, T.K. (2002). Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. Molecular cell *9*, 789-798.

Huang, M.J., Cheng, Y.C., Liu, C.R., Lin, S., and Liu, H.E. (2006). A small-molecule c-Myc inhibitor, 10058-F4, induces cell-cycle arrest, apoptosis, and myeloid differentiation of human acute myeloid leukemia. Experimental hematology *34*, 1480-1489.

Huang, Y.H., Yang, P.M., Chuah, Q.Y., Lee, Y.J., Hsieh, Y.F., Peng, C.W., and Chiu, S.J. (2014). Autophagy promotes radiation-induced senescence but inhibits bystander effects in human breast cancer cells. Autophagy *10*, 1212-1228.

Hubackova, S., Krejcikova, K., Bartek, J., and Hodny, Z. (2012). IL1- and TGFbeta-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'bystander senescence'. Aging (Albany NY) *4*, 932-951.

Hutchison, C.J. (2002). Lamins: building blocks or regulators of gene expression? Nat Rev Mol Cell Biol *3*, 848-858.

Hydbring, P., Bahram, F., Su, Y., Tronnersjo, S., Hogstrand, K., von der Lehr, N., Sharifi, H.R., Lilischkis, R., Hein, N., Wu, S.*, et al.* (2010). Phosphorylation by Cdk2 is required for Myc to repress Ras-induced senescence in cotransformation. P Natl Acad Sci USA *107*, 58-63.

Hydbring, P., Castell, A., and Larsson, L.G. (2017). MYC Modulation around the CDK2/p27/SKP2 Axis. Genes (Basel) *8*.

Iannello, A., Thompson, T.W., Ardolino, M., Lowe, S.W., and Raulet, D.H. (2013). p53-dependent chemokine production by senescent tumor cells supports NKG2D-dependent tumor elimination by natural killer cells. J Exp Med *210*, 2057-2069.

Ivanov, A., Pawlikowski, J., Manoharan, I., van Tuyn, J., Nelson, D.M., Rai, T.S., Shah, P.P., Hewitt, G., Korolchuk, V.I., Passos, J.F.*, et al.* (2013). Lysosome-mediated processing of chromatin in senescence. J Cell Biol *202*, 129-143.

Jacobs, J.F., Nierkens, S., Figdor, C.G., de Vries, I.J., and Adema, G.J. (2012). Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? Lancet Oncol *13*, e32-42.

Jaenicke, L.A., von Eyss, B., Carstensen, A., Wolf, E., Xu, W., Greifenberg, A.K., Geyer, M., Eilers, M., and Popov, N. (2016). Ubiquitin-Dependent Turnover of MYC Antagonizes MYC/PAF1C Complex Accumulation to Drive Transcriptional Elongation. Mol Cell *61*, 54-67.

Johnsen, J.I., Dyberg, C., Fransson, S., and Wickstrom, M. (2018). Molecular mechanisms and therapeutic targets in neuroblastoma. Pharmacol Res *131*, 164-176.

Kaplon, J., Zheng, L., Meissl, K., Chaneton, B., Selivanov, V.A., Mackay, G., van der Burg, S.H., Verdegaal, E.M., Cascante, M., Shlomi, T.*, et al.* (2013). A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature *498*, 109-112.

Kelly, K., Cochran, B.H., Stiles, C.D., and Leder, P. (1983). Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell *35*, 603-610.

Kerppola, T.K. (2006). Complementary methods for studies of protein interactions in living cells. Nat Methods *3*, 969-971.

Kiessling, A., Sperl, B., Hollis, A., Eick, D., and Berg, T. (2006). Selective inhibition of c-Myc/Max dimerization and DNA binding by small molecules. Chem Biol *13*, 745-751.

Kim, E.Y., Kim, A., Kim, S.K., and Chang, Y.S. (2017). MYC expression correlates with PD-L1 expression in non-small cell lung cancer. Lung Cancer *110*, 63-67.

Kim, J., Lee, J.H., and Iyer, V.R. (2008). Global identification of Myc target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. PLoS One *3*, e1798.

Kim, S.Y., Herbst, A., Tworkowski, K.A., Salghetti, S.E., and Tansey, W.P. (2003). Skp2 regulates Myc protein stability and activity. Molecular Cell *11*, 1177-1188.

Kohl, N.E., Kanda, N., Schreck, R.R., Bruns, G., Latt, S.A., Gilbert, F., and Alt, F.W. (1983). Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell *35*, 359-367.

Kojima, H., Kunimoto, H., Inoue, T., and Nakajima, K. (2012). The STAT3-IGFBP5 axis is critical for IL-6/gp130-induced premature senescence in human fibroblasts. Cell Cycle *11*, 730-739.

Kortlever, R.M., Sodir, N.M., Wilson, C.H., Burkhart, D.L., Pellegrinet, L., Brown Swigart, L., Littlewood, T.D., and Evan, G.I. (2017). Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. Cell *171*, 1301-1315 e1314.

Kosar, M., Bartkova, J., Hubackova, S., Hodny, Z., Lukas, J., and Bartek, J. (2011). Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16(ink4a). Cell Cycle *10*, 457-468.

Krtolica, A., Parrinello, S., Lockett, S., Desprez, P.Y., and Campisi, J. (2001). Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci U S A *98*, 12072-12077.

Kuilman, T., Michaloglou, C., Mooi, W.J., and Peeper, D.S. (2010). The essence of senescence. Genes Dev *24*, 2463-2479.

Kuilman, T., Michaloglou, C., Vredeveld, L.C., Douma, S., van Doorn, R., Desmet, C.J., Aarden, L.A., Mooi, W.J., and Peeper, D.S. (2008). Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell *133*, 1019-1031.

Kurland, J.F., and Tansey, W.P. (2008). Myc-mediated transcriptional repression by recruitment of histone deacetylase. Cancer Res *68*, 3624-3629.

Kurz, D.J., Decary, S., Hong, Y., and Erusalimsky, J.D. (2000). Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci *113 (Pt 20)*, 3613-3622.

Larsson, L.G. (2011). Cellular senescence--a barrier against tumor development? Seminars in cancer biology *21*, 347-348.

Larsson, L.G., and Henriksson, M.A. (2010). The Yin and Yang functions of the Myc oncoprotein in cancer development and as targets for therapy. Exp Cell Res *316*, 1429-1437.

Lee, B.Y., Han, J.A., Im, J.S., Morrone, A., Johung, K., Goodwin, E.C., Kleijer, W.J., DiMaio, D., and Hwang, E.S. (2006). Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell *5*, 187-195.

Lee, S., Jeong, S.Y., Lim, W.C., Kim, S., Park, Y.Y., Sun, X., Youle, R.J., and Cho, H. (2007). Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. J Biol Chem *282*, 22977-22983.

Leone, G., DeGregori, J., Sears, R., Jakoi, L., and Nevins, J.R. (1997). Myc and Ras collaborate in inducing accumulation of active cyclin E/Cdk2 and E2F. Nature *387*, 422-426.

Lessard, F., Igelmann, S., Trahan, C., Huot, G., Saint-Germain, E., Mignacca, L., Del Toro, N., Lopes-Paciencia, S., Le Calve, B., Montero, M.*, et al.* (2018). Senescence-associated ribosome biogenesis defects contributes to cell cycle arrest through the Rb pathway. Nat Cell Biol *20*, 789-799.

Levens, D. (2008). How the c-myc promoter works and why it sometimes does not. J Natl Cancer Inst Monogr, 41-43.

Li, Z., and Hann, S.R. (2009). The Myc-nucleophosmin-ARF network: a complex web unveiled. Cell Cycle *8*, 2703-2707.

Lin, C.Y., Loven, J., Rahl, P.B., Paranal, R.M., Burge, C.B., Bradner, J.E., Lee, T.I., and Young, R.A. (2012). Transcriptional amplification in tumor cells with elevated c-Myc. Cell *151*, 56-67.

Littlewood, T.D., Hancock, D.C., Danielian, P.S., Parker, M.G., and Evan, G.I. (1995). A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. Nucleic Acids Res *23*, 1686-1690.

Liu, H., He, Z., and Simon, H.U. (2014). Autophagy suppresses melanoma tumorigenesis by inducing senescence. Autophagy *10*, 372-373.

Liu, J., and Levens, D. (2006). Making myc. Curr Top Microbiol Immunol *302*, 1-32.

Liu, X., Tesfai, J., Evrard, Y.A., Dent, S.Y., and Martinez, E. (2003). c-Myc transformation domain recruits the human STAGA complex and requires TRRAP and GCN5 acetylase activity for transcription activation. J Biol Chem *278*, 20405-20412.

Lujambio, A., Akkari, L., Simon, J., Grace, D., Tschaharganeh, D.F., Bolden, J.E., Zhao, Z., Thapar, V., Joyce, J.A., Krizhanovsky, V.*, et al.* (2013). Non-cell-autonomous tumor suppression by p53. Cell *153*, 449-460.

Lutterbach, B., and Hann, S.R. (1994). Hierarchical phosphorylation at N-terminal transformation-sensitive sites in c-Myc protein is regulated by mitogens and in mitosis. Mol Cell Biol *14*, 5510-5522.

Maclean, K.H., Kastan, M.B., and Cleveland, J.L. (2007). Atm deficiency affects both apoptosis and proliferation to augment Myc-induced lymphomagenesis. Mol Cancer Res *5*, 705-711.

Maghazachi, A.A., Al-Aoukaty, A., and Schall, T.J. (1996). CC chemokines induce the generation of killer cells from CD56+ cells. Eur J Immunol *26*, 315-319.

Majeti, R., Chao, M.P., Alizadeh, A.A., Pang, W.W., Jaiswal, S., Gibbs, K.D., Jr., van Rooijen, N., and Weissman, I.L. (2009). CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell *138*, 286-299.

Mannava, S., Grachtchouk, V., Wheeler, L.J., Im, M., Zhuang, D., Slavina, E.G., Mathews, C.K., Shewach, D.S., and Nikiforov, M.A. (2008). Direct role of nucleotide metabolism in C-MYC-dependent proliferation of melanoma cells. Cell Cycle *7*, 2392-2400.

Manning, A.L., and Dyson, N.J. (2011). pRB, a tumor suppressor with a stabilizing presence. Trends Cell Biol *21*, 433-441.

Manning, B.D., and Toker, A. (2017). AKT/PKB Signaling: Navigating the Network. Cell *169*, 381-405.

Mar, F.A., Debnath, J., and Stohr, B.A. (2015). Autophagy-independent senescence and genome instability driven by targeted telomere dysfunction. Autophagy *11*, 527-537.

Martin-Subero, J.I., Odero, M.D., Hernandez, R., Cigudosa, J.C., Agirre, X., Saez, B., Sanz-Garcia, E., Ardanaz, M.T., Novo, F.J., Gascoyne, R.D.*, et al.* (2005). Amplification of IGH/MYC fusion in clinically aggressive IGH/BCL2-positive germinal center B-cell lymphomas. Genes Chromosomes Cancer *43*, 414-423.

Mason, D.X., Jackson, T.J., and Lin, A.W. (2004). Molecular signature of oncogenic ras-induced senescence. Oncogene *23*, 9238-9246.

Maya-Mendoza, A., Ostrakova, J., Kosar, M., Hall, A., Duskova, P., Mistrik, M., Merchut-Maya, J.M., Hodny, Z., Bartkova, J., Christensen, C.*, et al.* (2015). Myc and Ras oncogenes engage different energy metabolism programs and evoke distinct patterns of oxidative and DNA replication stress. Mol Oncol *9*, 601-616.

McKeown, M.R., and Bradner, J.E. (2014). Therapeutic strategies to inhibit MYC. Cold Spring Harbor perspectives in medicine *4*.

McMahon, S.B., Van Buskirk, H.A., Dugan, K.A., Copeland, T.D., and Cole, M.D. (1998). The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. Cell *94*, 363-374.

McMahon, S.B., Wood, M.A., and Cole, M.D. (2000). The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. Mol Cell Biol *20*, 556-562.

Melaiu, O., Mina, M., Chierici, M., Boldrini, R., Jurman, G., Romania, P., D'Alicandro, V., Benedetti, M.C., Castellano, A., Liu, T.*, et al.* (2017). PD-L1 Is a Therapeutic Target of the Bromodomain Inhibitor JQ1 and, Combined with HLA Class I, a Promising Prognostic Biomarker in Neuroblastoma. Clin Cancer Res *23*, 4462-4472.

Menssen, A., and Hermeking, H. (2002). Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes. Proc Natl Acad Sci U S A *99*, 6274-6279.

Meyer, N., and Penn, L.Z. (2008). Reflecting on 25 years with MYC. Nat Rev Cancer *8*, 976-990.

Michaloglou, C., Vredeveld, L.C., Soengas, M.S., Denoyelle, C., Kuilman, T., van der Horst, C.M., Majoor, D.M., Shay, J.W., Mooi, W.J., and Peeper, D.S. (2005). BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature *436*, 720-724.

Mihaylova, M.M., and Shaw, R.J. (2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat Cell Biol *13*, 1016-1023.

Milella, M., Falcone, I., Conciatori, F., Cesta Incani, U., Del Curatolo, A., Inzerilli, N., Nuzzo, C.M., Vaccaro, V., Vari, S., Cognetti, F.*, et al.* (2015). PTEN: Multiple Functions in Human Malignant Tumors. Front Oncol *5*, 24.

Miliani de Marval, P.L., Macias, E., Rounbehler, R., Sicinski, P., Kiyokawa, H., Johnson, D.G., Conti, C.J., and Rodriguez-Puebla, M.L. (2004). Lack of cyclin-dependent kinase 4 inhibits c-myc tumorigenic activities in epithelial tissues. Molecular and cellular biology *24*, 7538-7547.

Moasser, M.M. (2007). The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene *26*, 6469-6487.

Moiseeva, O., Bourdeau, V., Roux, A., Deschenes-Simard, X., and Ferbeyre, G. (2009). Mitochondrial dysfunction contributes to oncogene-induced senescence. Mol Cell Biol *29*, 4495-4507.

Molenaar, J.J., Ebus, M.E., Geerts, D., Koster, J., Lamers, F., Valentijn, L.J., Westerhout, E.M., Versteeg, R., and Caron, H.N. (2009). Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells. Proc Natl Acad Sci U S A *106*, 12968-12973.

Molenaar, J.J., Koster, J., Zwijnenburg, D.A., van Sluis, P., Valentijn, L.J., van der Ploeg, I., Hamdi, M., van Nes, J., Westerman, B.A., van Arkel, J.*, et al.* (2012). Sequencing of neuroblastoma identifies chromothripsis and defects in neuritogenesis genes. Nature *483*, 589-593.

Morrish, F., Neretti, N., Sedivy, J.M., and Hockenbery, D.M. (2008). The oncogene c-Myc coordinates regulation of metabolic networks to enable rapid cell cycle entry. Cell Cycle *7*, 1054-1066.

Mukhopadhyay, S., Readling, J., Cotter, P.D., Shrimpton, A.E., and Sidhu, J.S. (2005). Transformation of follicular lymphoma to Burkitt-like lymphoma within a single lymph node. Hum Pathol *36*, 571-575.

Munoz-Espin, D., Canamero, M., Maraver, A., Gomez-Lopez, G., Contreras, J., Murillo-Cuesta, S., Rodriguez-Baeza, A., Varela-Nieto, I., Ruberte, J., Collado, M.*, et al.* (2013). Programmed cell senescence during mammalian embryonic development. Cell *155*, 1104-1118.

Munoz-Espin, D., and Serrano, M. (2014). Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol *15*, 482-496.

Murphy, D.J., Junttila, M.R., Pouyet, L., Karnezis, A., Shchors, K., Bui, D.A., Brown-Swigart, L., Johnson, L., and Evan, G.I. (2008). Distinct thresholds govern Myc's biological output in vivo. Cancer cell *14*, 447-457.

Murre, C., McCaw, P.S., Vaessin, H., Caudy, M., Jan, L.Y., Jan, Y.N., Cabrera, C.V., Buskin, J.N., Hauschka, S.D., Lassar, A.B.*, et al.* (1989). Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. Cell *58*, 537-544.

Nardella, C., Clohessy, J.G., Alimonti, A., and Pandolfi, P.P. (2011). Pro-senescence therapy for cancer treatment. Nat Rev Cancer *11*, 503-511.

Narita, M., Narita, M., Krizhanovsky, V., Nunez, S., Chicas, A., Hearn, S.A., Myers, M.P., and Lowe, S.W. (2006). A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell *126*, 503-514.

Narita, M., Nunez, S., Heard, E., Narita, M., Lin, A.W., Hearn, S.A., Spector, D.L., Hannon, G.J., and Lowe, S.W. (2003). Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell *113*, 703-716.

Nau, M.M., Brooks, B.J., Battey, J., Sausville, E., Gazdar, A.F., Kirsch, I.R., McBride, O.W., Bertness, V., Hollis, G.F., and Minna, J.D. (1985). L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. Nature *318*, 69-73.

Neuzillet, C., Tijeras-Raballand, A., Cohen, R., Cros, J., Faivre, S., Raymond, E., and de Gramont, A. (2015). Targeting the TGFbeta pathway for cancer therapy. Pharmacol Ther *147*, 22-31.

Nie, Z., Hu, G., Wei, G., Cui, K., Yamane, A., Resch, W., Wang, R., Green, D.R., Tessarollo, L., Casellas, R.*, et al.* (2012). c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell *151*, 68-79.

Nilsson, J.A., and Cleveland, J.L. (2003). Myc pathways provoking cell suicide and cancer. Oncogene *22*, 9007-9021.

Oble, D.A., Loewe, R., Yu, P., and Mihm, M.C., Jr. (2009). Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma. Cancer Immun *9*, 3.

Orian, A., van Steensel, B., Delrow, J., Bussemaker, H.J., Li, L., Sawado, T., Williams, E., Loo, L.W., Cowley, S.M., Yost, C.*, et al.* (2003). Genomic binding by the Drosophila Myc, Max, Mad/Mnt transcription factor network. Genes Dev *17*, 1101-1114.

Ostrand-Rosenberg, S., and Fenselau, C. (2018). Myeloid-Derived Suppressor Cells: Immune-Suppressive Cells That Impair Antitumor Immunity and Are Sculpted by Their Environment. J Immunol *200*, 422-431.

Park, J., Wood, M.A., and Cole, M.D. (2002). BAF53 forms distinct nuclear complexes and functions as a critical c-Myc-interacting nuclear cofactor for oncogenic transformation. Mol Cell Biol *22*, 1307-1316.

Parry, D., Bates, S., Mann, D.J., and Peters, G. (1995). Lack of cyclin D-Cdk complexes in Rb-negative cells correlates with high levels of p16INK4/MTS1 tumour suppressor gene product. EMBO J *14*, 503-511.

Passos, J.F., Nelson, G., Wang, C., Richter, T., Simillion, C., Proctor, C.J., Miwa, S., Olijslagers, S., Hallinan, J., Wipat, A.*, et al.* (2010). Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol Syst Biol *6*, 347.

Peter, M.E., Hadji, A., Murmann, A.E., Brockway, S., Putzbach, W., Pattanayak, A., and Ceppi, P. (2015). The role of CD95 and CD95 ligand in cancer. Cell Death Differ *22*, 549-559.

Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W., and Vogelstein, B. (1997). A model for p53-induced apoptosis. Nature *389*, 300-305.

Prendergast, G.C., Lawe, D., and Ziff, E.B. (1991). Association of Myn, the murine homolog of max, with c-Myc stimulates methylation-sensitive DNA binding and ras cotransformation. Cell *65*, 395-407.

Prochownik, E.V., and Vogt, P.K. (2010). Therapeutic Targeting of Myc. Genes Cancer *1*, 650-659.

Pusapati, R.V., Rounbehler, R.J., Hong, S., Powers, J.T., Yan, M., Kiguchi, K., McArthur, M.J., Wong, P.K., and Johnson, D.G. (2006). ATM promotes apoptosis and suppresses tumorigenesis in response to Myc. Proc Natl Acad Sci U S A *103*, 1446-1451.

Qing, G., Li, B., Vu, A., Skuli, N., Walton, Z.E., Liu, X., Mayes, P.A., Wise, D.R., Thompson, C.B., Maris, J.M.*, et al.* (2012). ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. Cancer Cell *22*, 631-644.

Rahl, P.B., Lin, C.Y., Seila, A.C., Flynn, R.A., McCuine, S., Burge, C.B., Sharp, P.A., and Young, R.A. (2010). c-Myc regulates transcriptional pause release. Cell *141*, 432-445.

Rakhra, K., Bachireddy, P., Zabuawala, T., Zeiser, R., Xu, L., Kopelman, A., Fan, A.C., Yang, Q., Braunstein, L., Crosby, E.*, et al.* (2010). CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. Cancer cell *18*, 485-498.

Ratner, N., and Miller, S.J. (2015). A RASopathy gene commonly mutated in cancer: the neurofibromatosis type 1 tumour suppressor. Nat Rev Cancer *15*, 290-301.

Rayess, H., Wang, M.B., and Srivatsan, E.S. (2012). Cellular senescence and tumor suppressor gene p16. Int J Cancer *130*, 1715-1725.

Reed, J.C., Alpers, J.D., Nowell, P.C., and Hoover, R.G. (1986). Sequential expression of protooncogenes during lectin-stimulated mitogenesis of normal human lymphocytes. Proc Natl Acad Sci U S A *83*, 3982-3986.

Remy, I., and Michnick, S.W. (2006). A highly sensitive protein-protein interaction assay based on Gaussia luciferase. Nat Methods *3*, 977-979.

Robert, C., Long, G.V., Brady, B., Dutriaux, C., Maio, M., Mortier, L., Hassel, J.C., Rutkowski, P., McNeil, C., Kalinka-Warzocha, E.*, et al.* (2015). Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med *372*, 320-330.

Rous, P. (1910). A Transmissible Avian Neoplasm. (Sarcoma of the Common Fowl.). J Exp Med *12*, 696-705.

Rous, P. (1911). A Sarcoma of the Fowl Transmissible by an Agent Separable from the Tumor Cells. J Exp Med *13*, 397-411.

Ruhland, M.K., Loza, A.J., Capietto, A.H., Luo, X., Knolhoff, B.L., Flanagan, K.C., Belt, B.A., Alspach, E., Leahy, K., Luo, J.*, et al.* (2016). Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. Nat Commun *7*, 11762.

Ruscetti, M., Leibold, J., Bott, M.J., Fennell, M., Kulick, A., Salgado, N.R., Chen, C.C., Ho, Y.J., Sanchez-Rivera, F.J., Feucht, J.*, et al.* (2018). NK cell-mediated cytotoxicity contributes to tumor control by a cytostatic drug combination. Science *362*, 1416-1422.

Sabo, A., Kress, T.R., Pelizzola, M., de Pretis, S., Gorski, M.M., Tesi, A., Morelli, M.J., Bora, P., Doni, M., Verrecchia, A.*, et al.* (2014). Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. Nature *511*, 488-492.

Sankar, N., Kadeppagari, R.K., and Thimmapaya, B. (2009). c-Myc-induced aberrant DNA synthesis and activation of DNA damage response in p300 knockdown cells. J Biol Chem *284*, 15193-15205.

Schachter, J., Ribas, A., Long, G.V., Arance, A., Grob, J.J., Mortier, L., Daud, A., Carlino, M.S., McNeil, C., Lotem, M.*, et al.* (2017). Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet *390*, 1853-1862.

Schasfoort, R.B.M., and Tudos, A.J. (2008). Handbook of surface plasmon resonance (Cambridge, UK: RSC Pub.).

Schlee, M., Holzel, M., Bernard, S., Mailhammer, R., Schuhmacher, M., Reschke, J., Eick, D., Marinkovic, D., Wirth, T., Rosenwald, A.*, et al.* (2007). C-myc activation impairs the NF-kappaB and the interferon response: implications for the pathogenesis of Burkitt's lymphoma. Int J Cancer *120*, 1387-1395.

Schlosser, I., Holzel, M., Murnseer, M., Burtscher, H., Weidle, U.H., and Eick, D. (2003). A role for c-Myc in the regulation of ribosomal RNA processing. Nucleic Acids Res *31*, 6148-6156.

Schmidt, E.V. (2004). The role of c-myc in regulation of translation initiation. Oncogene *23*, 3217-3221.

Schuhmacher, M., Kohlhuber, F., Holzel, M., Kaiser, C., Burtscher, H., Jarsch, M., Bornkamm, G.W., Laux, G., Polack, A., Weidle, U.H.*, et al.* (2001). The transcriptional program of a human B cell line in response to Myc. Nucleic Acids Res *29*, 397-406.

Schuhmacher, M., Staege, M.S., Pajic, A., Polack, A., Weidle, U.H., Bornkamm, G.W., Eick, D., and Kohlhuber, F. (1999). Control of cell growth by c-Myc in the absence of cell division. Curr Biol *9*, 1255-1258.

Schuijers, J., Manteiga, J.C., Weintraub, A.S., Day, D.S., Zamudio, A.V., Hnisz, D., Lee, T.I., and Young, R.A. (2018). Transcriptional Dysregulation of MYC Reveals Common Enhancer-Docking Mechanism. Cell Rep *23*, 349-360.

Schumacher, T.N., and Schreiber, R.D. (2015). Neoantigens in cancer immunotherapy. Science *348*, 69-74.

Schwab, M., Alitalo, K., Klempnauer, K.H., Varmus, H.E., Bishop, J.M., Gilbert, F., Brodeur, G., Goldstein, M., and Trent, J. (1983). Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature *305*, 245-248.

Sears, R., Leone, G., DeGregori, J., and Nevins, J.R. (1999). Ras enhances Myc protein stability. Mol Cell *3*, 169-179.

Sears, R., Nuckolls, F., Haura, E., Taya, Y., Tamai, K., and Nevins, J.R. (2000). Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes Dev *14*, 2501-2514.

Sears, R., Ohtani, K., and Nevins, J.R. (1997). Identification of positively and negatively acting elements regulating expression of the E2F2 gene in response to cell growth signals. Mol Cell Biol *17*, 5227-5235.

Seeger, R.C., Brodeur, G.M., Sather, H., Dalton, A., Siegel, S.E., Wong, K.Y., and Hammond, D. (1985). Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med *313*, 1111-1116.

Seitz, V., Butzhammer, P., Hirsch, B., Hecht, J., Gutgemann, I., Ehlers, A., Lenze, D., Oker, E., Sommerfeld, A., von der Wall, E.*, et al.* (2011). Deep sequencing of MYC DNA-binding sites in Burkitt lymphoma. PLoS One *6*, e26837.

Seo, H.R., Kim, J., Bae, S., Soh, J.W., and Lee, Y.S. (2008). Cdk5-mediated phosphorylation of c-Myc on Ser-62 is essential in transcriptional activation of cyclin B1 by cyclin G1. J Biol Chem *283*, 15601-15610.

Seoane, J., Le, H.V., and Massague, J. (2002). Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. Nature *419*, 729-734.

Seoane, J., Pouponnot, C., Staller, P., Schader, M., Eilers, M., and Massague, J. (2001). TGFbeta influences Myc, Miz-1 and Smad to control the CDK inhibitor p15INK4b. Nat Cell Biol *3*, 400-408.

Serrano, M., Hannon, G.J., and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature *366*, 704-707.

Seth, A., Alvarez, E., Gupta, S., and Davis, R.J. (1991). A phosphorylation site located in the NH2-terminal domain of c-Myc increases transactivation of gene expression. J Biol Chem *266*, 23521-23524.

Sfeir, A., and de Lange, T. (2012). Removal of shelterin reveals the telomere end-protection problem. Science *336*, 593-597.

Shain, A.H., and Bastian, B.C. (2016). From melanocytes to melanomas. Nat Rev Cancer *16*, 345-358.

Sharpless, N.E., and Sherr, C.J. (2015). Forging a signature of in vivo senescence. Nat Rev Cancer *15*, 397-408.

Shaulian, E., and Karin, M. (2001). AP-1 in cell proliferation and survival. Oncogene *20*, 2390-2400.

Shay, J.W., and Wright, W.E. (2005). Senescence and immortalization: role of telomeres and telomerase. Carcinogenesis *26*, 867-874.

Shin, S.Y., Rath, O., Choo, S.M., Fee, F., McFerran, B., Kolch, W., and Cho, K.H. (2009). Positive- and negative-feedback regulations coordinate the dynamic behavior of the Ras-Raf-MEK-ERK signal transduction pathway. J Cell Sci *122*, 425-435.

Siegel, R.L., Miller, K.D., and Jemal, A. (2018). Cancer statistics, 2018. CA Cancer J Clin *68*, 7-30.

Soderberg, O., Gullberg, M., Jarvius, M., Ridderstrale, K., Leuchowius, K.J., Jarvius, J., Wester, K., Hydbring, P., Bahram, F., Larsson, L.G.*, et al.* (2006). Direct observation of individual endogenous protein complexes in situ by proximity ligation. Nat Methods *3*, 995-1000.

Sonenberg, N., and Hinnebusch, A.G. (2009). Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell *136*, 731-745.

Sosman, J.A., Kim, K.B., Schuchter, L., Gonzalez, R., Pavlick, A.C., Weber, J.S., McArthur, G.A., Hutson, T.E., Moschos, S.J., Flaherty, K.T.*, et al.* (2012). Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med *366*, 707-714.

Spranger, S., Bao, R., and Gajewski, T.F. (2015). Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature *523*, 231-235.

Srinivasan, S.V., Dominguez-Sola, D., Wang, L.C., Hyrien, O., and Gautier, J. (2013). Cdc45 is a critical effector of myc-dependent DNA replication stress. Cell Rep *3*, 1629-1639.

Staller, P., Peukert, K., Kiermaier, A., Seoane, J., Lukas, J., Karsunky, H., Moroy, T., Bartek, J., Massague, J., Hanel, F.*, et al.* (2001). Repression of p15(INK4b) expression by Myc through association with Miz-1. Nat Cell Biol *3*, 392-399.

Steele, C.W., Karim, S.A., Leach, J.D.G., Bailey, P., Upstill-Goddard, R., Rishi, L., Foth, M., Bryson, S., McDaid, K., Wilson, Z.*, et al.* (2016). CXCR2 Inhibition Profoundly Suppresses Metastases and Augments Immunotherapy in Pancreatic Ductal Adenocarcinoma. Cancer Cell *29*, 832-845.

Steinman, R.M., and Hemmi, H. (2006). Dendritic cells: translating innate to adaptive immunity. Curr Top Microbiol Immunol *311*, 17-58.

Stine, Z.E., Walton, Z.E., Altman, B.J., Hsieh, A.L., and Dang, C.V. (2015). MYC, Metabolism, and Cancer. Cancer Discov *5*, 1024-1039.

Sullivan, R.J., and Flaherty, K.T. (2013). Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer *49*, 1297-1304.

Swanson, E.C., Manning, B., Zhang, H., and Lawrence, J.B. (2013). Higher-order unfolding of satellite heterochromatin is a consistent and early event in cell senescence. J Cell Biol *203*, 929-942.

Sweis, R.F., Spranger, S., Bao, R., Paner, G.P., Stadler, W.M., Steinberg, G., and Gajewski, T.F. (2016). Molecular Drivers of the Non-T-cell-Inflamed Tumor Microenvironment in Urothelial Bladder Cancer. Cancer Immunol Res *4*, 563-568.

Tabor, V., Bocci, M., Alikhani, N., Kuiper, R., and Larsson, L.G. (2014). MYC synergizes with activated BRAFV600E in mouse lung tumor development by suppressing senescence. Cancer Res *74*, 4222-4229.

Tai, H., Wang, Z., Gong, H., Han, X., Zhou, J., Wang, X., Wei, X., Ding, Y., Huang, N., Qin, J.*, et al.* (2017). Autophagy impairment with lysosomal and mitochondrial dysfunction is an important characteristic of oxidative stress-induced senescence. Autophagy *13*, 99-113.

Takahashi, A., Ohtani, N., and Hara, E. (2007). Irreversibility of cellular senescence: dual roles of p16INK4a/Rb-pathway in cell cycle control. Cell Div *2*, 10.

Takasaka, N., Araya, J., Hara, H., Ito, S., Kobayashi, K., Kurita, Y., Wakui, H., Yoshii, Y., Yumino, Y., Fujii, S.*, et al.* (2014). Autophagy induction by SIRT6 through attenuation of insulin-like growth factor

signaling is involved in the regulation of human bronchial epithelial cell senescence. J Immunol *192*, 958-968.

TCGA (The Cancer Genome Atlas). https://cancergenome.nih.gov/.

Thomas, L.R., and Tansey, W.P. (2011). Proteolytic control of the oncoprotein transcription factor Myc. Adv Cancer Res *110*, 77-106.

Toso, A., Revandkar, A., Di Mitri, D., Guccini, I., Proietti, M., Sarti, M., Pinton, S., Zhang, J., Kalathur, M., Civenni, G.*, et al.* (2014). Enhancing chemotherapy efficacy in Pten-deficient prostate tumors by activating the senescence-associated antitumor immunity. Cell Rep *9*, 75-89.

Tumeh, P.C., Harview, C.L., Yearley, J.H., Shintaku, I.P., Taylor, E.J., Robert, L., Chmielowski, B., Spasic, M., Henry, G., Ciobanu, V.*, et al.* (2014). PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature *515*, 568-571.

Vafa, O., Wade, M., Kern, S., Beeche, M., Pandita, T.K., Hampton, G.M., and Wahl, G.M. (2002). c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. Mol Cell *9*, 1031-1044.

van Deursen, J.M. (2014). The role of senescent cells in ageing. Nature *509*, 439-446.

van Riggelen, J., Yetil, A., and Felsher, D.W. (2010). MYC as a regulator of ribosome biogenesis and protein synthesis. Nat Rev Cancer *10*, 301-309.

Vennstrom, B., Sheiness, D., Zabielski, J., and Bishop, J.M. (1982). Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29. J Virol *42*, 773-779.

Vilgelm, A., and Richmond, A. (2015). Combined therapies that induce senescence and stabilize p53 block melanoma growth and prompt antitumor immune responses. Oncoimmunology *4*, e1009299.

Vilgelm, A.E., Johnson, C.A., Prasad, N., Yang, J., Chen, S.C., Ayers, G.D., Pawlikowski, J.S., Raman, D., Sosman, J.A., Kelley, M.*, et al.* (2016). Connecting the Dots: Therapy-Induced Senescence and a Tumor-Suppressive Immune Microenvironment. J Natl Cancer Inst *108*, djv406.

Vivier, E., Raulet, D.H., Moretta, A., Caligiuri, M.A., Zitvogel, L., Lanier, L.L., Yokoyama, W.M., and Ugolini, S. (2011). Innate or adaptive immunity? The example of natural killer cells. Science *331*, 44-49.

Vogler, M., Hamali, H.A., Sun, X.M., Bampton, E.T., Dinsdale, D., Snowden, R.T., Dyer, M.J., Goodall, A.H., and Cohen, G.M. (2011). BCL2/BCL-X(L) inhibition induces apoptosis, disrupts cellular calcium homeostasis, and prevents platelet activation. Blood *117*, 7145-7154.

von der Lehr, N., Johansson, S., Wu, S., Bahram, F., Castell, A., Cetinkaya, C., Hydbring, P., Weidung, I., Nakayama, K., Nakayama, K.I.*, et al.* (2003). The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. Mol Cell *11*, 1189-1200.

Wagle, N., Emery, C., Berger, M.F., Davis, M.J., Sawyer, A., Pochanard, P., Kehoe, S.M., Johannessen, C.M., Macconaill, L.E., Hahn, W.C.*, et al.* (2011). Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. J Clin Oncol *29*, 3085-3096.

Wagle, N., Van Allen, E.M., Treacy, D.J., Frederick, D.T., Cooper, Z.A., Taylor-Weiner, A., Rosenberg, M., Goetz, E.M., Sullivan, R.J., Farlow, D.N.*, et al.* (2014). MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. Cancer Discov *4*, 61-68.

Wajant, H., Pfizenmaier, K., and Scheurich, P. (2003). Tumor necrosis factor signaling. Cell Death Differ *10*, 45-65.

Walz, S., Lorenzin, F., Morton, J., Wiese, K.E., von Eyss, B., Herold, S., Rycak, L., Dumay-Odelot, H., Karim, S., Bartkuhn, M.*, et al.* (2014). Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. Nature *511*, 483-487.

Wan, J., Benkdane, M., Alons, E., Lotersztajn, S., and Pavoine, C. (2014). M2 kupffer cells promote hepatocyte senescence: an IL-6-dependent protective mechanism against alcoholic liver disease. Am J Pathol *184*, 1763-1772.

Wang, E. (1995). Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. Cancer Res *55*, 2284-2292.

Wang, H., Bauzon, F., Ji, P., Xu, X., Sun, D., Locker, J., Sellers, R.S., Nakayama, K., Nakayama, K.I., Cobrinik, D.*, et al.* (2010). Skp2 is required for survival of aberrantly proliferating Rb1-deficient cells and for tumorigenesis in Rb1+/- mice. Nat Genet *42*, 83-88.

Wang, H., Mannava, S., Grachtchouk, V., Zhuang, D., Soengas, M.S., Gudkov, A.V., Prochownik, E.V., and Nikiforov, M.A. (2008). c-Myc depletion inhibits proliferation of human tumor cells at various stages of the cell cycle. Oncogene *27*, 1905-1915.

Wang, J., Geesman, G.J., Hostikka, S.L., Atallah, M., Blackwell, B., Lee, E., Cook, P.J., Pasaniuc, B., Shariat, G., Halperin, E.*, et al.* (2011). Inhibition of activated pericentromeric SINE/Alu repeat transcription in senescent human adult stem cells reinstates self-renewal. Cell Cycle *10*, 3016-3030.

Wang, Y., Wang, X.D., Lapi, E., Sullivan, A., Jia, W., He, Y.W., Ratnayaka, I., Zhong, S., Goldin, R.D., Goemans, C.G.*, et al.* (2012). Autophagic activity dictates the cellular response to oncogenic RAS. Proc Natl Acad Sci U S A *109*, 13325-13330.

Weber, J., Mandala, M., Del Vecchio, M., Gogas, H.J., Arance, A.M., Cowey, C.L., Dalle, S., Schenker, M., Chiarion-Sileni, V., Marquez-Rodas, I.*, et al.* (2017). Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. N Engl J Med *377*, 1824-1835.

Weber, J.S., D'Angelo, S.P., Minor, D., Hodi, F.S., Gutzmer, R., Neyns, B., Hoeller, C., Khushalani, N.I., Miller, W.H., Jr., Lao, C.D.*, et al.* (2015). Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol *16*, 375-384.

Weinberg, R.A. (1995). The retinoblastoma protein and cell cycle control. Cell *81*, 323-330.

Welsh, S.J., Rizos, H., Scolyer, R.A., and Long, G.V. (2016). Resistance to combination BRAF and MEK inhibition in metastatic melanoma: Where to next? Eur J Cancer *62*, 76-85.

Whitfield, J.R., Beaulieu, M.E., and Soucek, L. (2017). Strategies to Inhibit Myc and Their Clinical Applicability. Front Cell Dev Biol *5*, 10.

Wiegering, A., Uthe, F.W., Jamieson, T., Ruoss, Y., Huttenrauch, M., Kuspert, M., Pfann, C., Nixon, C., Herold, S., Walz, S.*, et al.* (2015). Targeting translation initiation bypasses signaling crosstalk mechanisms that maintain high MYC levels in colorectal cancer. Cancer Discov.

Wierstra, I., and Alves, J. (2008). The c-myc promoter: still MysterY and challenge. Adv Cancer Res *99*, 113-333.

Wiley, C.D., and Campisi, J. (2016). From Ancient Pathways to Aging Cells-Connecting Metabolism and Cellular Senescence. Cell Metab *23*, 1013-1021.

Wiley, C.D., Velarde, M.C., Lecot, P., Liu, S., Sarnoski, E.A., Freund, A., Shirakawa, K., Lim, H.W., Davis, S.S., Ramanathan, A.*, et al.* (2016). Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. Cell Metab *23*, 303-314.

Wilmott, J.S., Long, G.V., Howle, J.R., Haydu, L.E., Sharma, R.N., Thompson, J.F., Kefford, R.F., Hersey, P., and Scolyer, R.A. (2012). Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res *18*, 1386-1394.

Wolfe, A.L., Singh, K., Zhong, Y., Drewe, P., Rajasekhar, V.K., Sanghvi, V.R., Mavrakis, K.J., Jiang, M., Roderick, J.E., Van der Meulen, J.*, et al.* (2014). RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. Nature *513*, 65-70.

Wood, M.A., McMahon, S.B., and Cole, M.D. (2000). An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. Mol Cell *5*, 321-330.

Wu, C.H., van Riggelen, J., Yetil, A., Fan, A.C., Bachireddy, P., and Felsher, D.W. (2007). Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. Proc Natl Acad Sci U S A *104*, 13028-13033.

Wu, S., Cetinkaya, C., Munoz-Alonso, M.J., von der Lehr, N., Bahram, F., Beuger, V., Eilers, M., Leon, J., and Larsson, L.G. (2003). Myc represses differentiation-induced p21CIP1 expression via Miz-1-dependent interaction with the p21 core promoter. Oncogene *22*, 351-360.

Xu, Y., Poggio, M., Jin, H.Y., Shi, Z., Forester, C.M., Wang, Y., Stumpf, C.R., Xue, L., Devericks, E., So, L.*, et al.* (2019). Translation control of the immune checkpoint in cancer and its therapeutic targeting. Nat Med *25*, 301-311.

Xue, W., Zender, L., Miething, C., Dickins, R.A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C., and Lowe, S.W. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature *445*, 656-660.

Yaguchi, T., Aida, S., Kaul, S.C., and Wadhwa, R. (2007). Involvement of mortalin in cellular senescence from the perspective of its mitochondrial import, chaperone, and oxidative stress management functions. Ann N Y Acad Sci *1100*, 306-311.

Yamaki, T., Suenaga, Y., Iuchi, T., Alagu, J., Takatori, A., Itami, M., Araki, A., Ohira, M., Inoue, M., Kageyama, H.*, et al.* (2013). Temozolomide suppresses MYC via activation of TAp63 to inhibit progression of human glioblastoma. Sci Rep *3*, 1160.

Yeh, E., Cunningham, M., Arnold, H., Chasse, D., Monteith, T., Ivaldi, G., Hahn, W.C., Stukenberg, P.T., Shenolikar, S., Uchida, T.*, et al.* (2004). A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nat Cell Biol *6*, 308-318.

Yin, X., Giap, C., Lazo, J.S., and Prochownik, E.V. (2003). Low molecular weight inhibitors of Myc-Max interaction and function. Oncogene *22*, 6151-6159.

Youle, R.J., and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol *9*, 47-59.

Young, A.R., Narita, M., Ferreira, M., Kirschner, K., Sadaie, M., Darot, J.F., Tavare, S., Arakawa, S., Shimizu, S., Watt, F.M.*, et al.* (2009). Autophagy mediates the mitotic senescence transition. Genes Dev *23*, 798-803.

Young, S.L., Diolaiti, D., Conacci-Sorrell, M., Ruiz-Trillo, I., Eisenman, R.N., and King, N. (2011). Premetazoan ancestry of the Myc-Max network. Mol Biol Evol *28*, 2961-2971.

Yousefzadeh, M.J., Zhu, Y., McGowan, S.J., Angelini, L., Fuhrmann-Stroissnigg, H., Xu, M., Ling, Y.Y., Melos, K.I., Pirtskhalava, T., Inman, C.L.*, et al.* (2018). Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine *36*, 18-28.

Yuneva, M., Zamboni, N., Oefner, P., Sachidanandam, R., and Lazebnik, Y. (2007). Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. J Cell Biol *178*, 93-105.

Zamai, L., Ahmad, M., Bennett, I.M., Azzoni, L., Alnemri, E.S., and Perussia, B. (1998). Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. J Exp Med *188*, 2375-2380.

Zaretsky, J.M., Garcia-Diaz, A., Shin, D.S., Escuin-Ordinas, H., Hugo, W., Hu-Lieskovan, S., Torrejon, D.Y., Abril-Rodriguez, G., Sandoval, S., Barthly, L.*, et al.* (2016). Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N Engl J Med *375*, 819-829.

Zdanov, S., Remacle, J., and Toussaint, O. (2006). Establishment of H2O2-induced premature senescence in human fibroblasts concomitant with increased cellular production of H2O2. Ann N Y Acad Sci *1067*, 210-216.

Zhang, R., Chen, W., and Adams, P.D. (2007). Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol *27*, 2343-2358.

Zhang, R., Poustovoitov, M.V., Ye, X., Santos, H.A., Chen, W., Daganzo, S.M., Erzberger, J.P., Serebriiskii, I.G., Canutescu, A.A., Dunbrack, R.L.*, et al.* (2005). Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell *8*, 19-30.

Zhu, H., Bengsch, F., Svoronos, N., Rutkowski, M.R., Bitler, B.G., Allegrezza, M.J., Yokoyama, Y., Kossenkov, A.V., Bradner, J.E., Conejo-Garcia, J.R.*, et al.* (2016a). BET Bromodomain Inhibition Promotes Anti-tumor Immunity by Suppressing PD-L1 Expression. Cell Rep *16*, 2829-2837.

Zhu, Y., Doornebal, E.J., Pirtskhalava, T., Giorgadze, N., Wentworth, M., Fuhrmann-Stroissnigg, H., Niedernhofer, L.J., Robbins, P.D., Tchkonia, T., and Kirkland, J.L. (2017). New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. Aging (Albany NY) *9*, 955-963.

Zhu, Y., Tchkonia, T., Fuhrmann-Stroissnigg, H., Dai, H.M., Ling, Y.Y., Stout, M.B., Pirtskhalava, T., Giorgadze, N., Johnson, K.O., Giles, C.B.*, et al.* (2016b). Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell *15*, 428-435.

Zhu, Y., Tchkonia, T., Pirtskhalava, T., Gower, A.C., Ding, H., Giorgadze, N., Palmer, A.K., Ikeno, Y., Hubbard, G.B., Lenburg, M.*, et al.* (2015). The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell *14*, 644-658.

Zhuang, D., Mannava, S., Grachtchouk, V., Tang, W.H., Patil, S., Wawrzyniak, J.A., Berman, A.E., Giordano, T.J., Prochownik, E.V., Soengas, M.S.*, et al.* (2008). C-MYC overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. Oncogene *27*, 6623-6634.

Zwerschke, W., Mazurek, S., Stockl, P., Hutter, E., Eigenbrodt, E., and Jansen-Durr, P. (2003). Metabolic analysis of senescent human fibroblasts reveals a role for AMP in cellular senescence. Biochem J *376*, 403-411.